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THE EMBRYOLOGY OF FULGUR: A STUDY OF THE INFLUENCE OF YOLK ON DEVELOPMENT.

BY EDWIN G. CONKLIN,
PROFESSOR OF ZOOLOGY, UNIVERSITY OF PENNSYLVANIA.

I. INTRODUCTION.

1. GENERAL.

It is generally believed that the accumulation of yolk in the eggs of certain animals has greatly modified their original manner of development. For example, selachians, reptiles and birds are probably derived from animals in which the eggs contained much less yolk than at present in these classes, and in which the development was different in many respects from that which now obtains among these forms. The effect of the loss of yolk upon development has been studied experimentally by Morgan (1893), but the influence of an increase in the amount of yolk, while the protoplasmic portion of the egg remains the same, has not been studied experimentally, and may perhaps lie beyond the test of experiment.[1] Such experiments, however, nature has performed in several different phyla of animals, but nowhere are natural conditions more favorable for a study of the influence of yolk on development than among the mollusks. Brooks (1879) long ago called attention to this fact, though it has not been reinvestigated in the light of the modern cellular study of development.

The comparison of the development of large eggs with that of small ones, with especial reference to the organization of the egg and the history of the cleavage cells, should be of considerable general interest. Does a great accumulation of yolk change the localization of morphogenetic processes and substances in the egg? Does it alter the developmental history and destiny of the blastomeres? Is the yolk itself localized in any definite germinal region of the egg? In what manner does a great mass of yolk alter the gastrulation and later embryonic development? These are questions upon which a detailed comparison of the normal development of large and of small eggs might be expected to shed some light. Undoubtedly experiments, if they could be successfully performed, would be of great value in answering these questions, but in the case of the gasteropods which I have studied I have not found it possible to test these questions experimentally.

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[1] The fusion of different eggs to form giants, such as occurs abnormally in certain cases, is not a case in point, since the relative amount of yolk and protoplasm remains the same in the fused eggs as in the single ones.

The development of gasteropods is extraordinarily varied in respect to the manner of nourishing the embryo and larva. In some cases, and these presumably the more primitive, the eggs are small and contain little yolk and the free swimming larvæ (veligers) are set free at an early age to shift for themselves (*e.g.*, *Crepidula plana*, *Patella*, *Acmæa*, etc.). In others the eggs contain a larger quantity of yolk and the free-swimming life is correspondingly reduced (*e.g.*, *Crepidula fornicata*, *Illyonassa*, etc.). In still others the eggs contain a yet greater quantity of yolk and the veliger stage is passed entirely within the egg capsules, the young escaping in practically an adult condition (*e.g.*, *Crepidula convexa* and *adunca*, *Urosalpinx*, *Sycotypus*, *Fulgur*, etc.).

A most curious and interesting method by which nutriment is supplied to the embryo is found in those gasteropods in which a large number of relatively small eggs is laid, only a few of which develop, the others being eaten as food by the developing embryos (*e.g.*, *Purpura*, *Buccinum*, *Fasciolaria*, *Neritina*, etc.).

For the purposes of the present study the only instances which we shall consider are those in which the food for the developing embryos is contained within the egg in the form of yolk. The early development of a considerable number of gasteropods is now well known, but most of these belong to that group having relatively small eggs. It has seemed to me worth while to compare with these the development of the largest gasteropod egg of which I have any knowledge, viz., that of *Fulgur carica*. The eggs of this species are about one-sixteenth of an inch in diameter; those of a closely allied form, *Sycotypus canalliculatus*, are about one-twenty-fifth of an inch in diameter. The eggs of *Fulgur* are about thirteen times the diameter and about 2,200 times the volume of the eggs of *Crepidula plana*, with which I shall particularly compare them. I have also studied several other species in which the eggs are intermediate in size between these two extremes, as shown by the following table:

Fulgur carica, ...	1,700 μ in diameter.
Sycotypus canalliculatus, ...	1,000 μ "
Crepidula adunca	410 μ "
" *convexa*, ...	280 μ "
" *fornicata*, ...	182 μ "
" *plana*, ...	136 μ "

The great difference in the sizes of these eggs is not due to a proportionate difference in all their constituents, viz., protoplasm as well as yolk, but almost entirely to the varying amounts of yolk which they contain, and this fact suggests the possibility of examining the influence of yolk upon development by comparing the development of one of the largest gasteropod eggs with one of the smallest.

A paper on the embryology of *Fulgur* was published by McMurrich (1886) more than twenty years ago. This paper deals in a general way with the development of this genus, questions of cell lineage and egg organization being at that time almost unknown. Further reference to McMurrich's work will be made throughout the course of this paper. A subsequent paper by the same author (1896) deals with the yolk lobe and centrosome of *Fulgur*. Some features in the development of this form were also considered by me (1897) in a paper dealing more particularly with *Crepidula*. Other than this the development of *Fulgur* has not been made the subject of any detailed study.

2. BREEDING HABITS.

For the sake of completeness I shall here mention some observations on the breeding habits of *Fulgur* and *Sycotypus*. In both of these forms the eggs are laid in peculiar disk-shaped or lenticular capsules which are attached in a series along a central cord. With the exception of the first few capsules formed, which are small and usually contain no eggs, each capsule contains from ten to twenty eggs embedded in a gelatinous substance which fills the capsules.

The method of forming the peculiar string of capsules characteristic of these genera is sufficiently interesting to merit a brief description. During the process of egg-laying, the foot of the female is contracted and partially withdrawn within the shell. A mucous-like secretion is then poured out from the nidamental organ through the folds of the partially contracted foot, and this secretion is applied to some solid object to which it adheres, or it is merely buried in the sand. In the case of *Fulgur* this first formed portion of the "string" is buried deeply in the sand; in certain specimens of *Sycotypus* which I kept in a wooden car, or float, the egg string was attached to the boards of the car. The first formed capsules on the string are small, far apart and contain no eggs. Later formed capsules increase in size and in the number of eggs contained in each, and successive capsules lie closer together on the string. During the process of laying the female is quiescent, and is usually buried beneath the surface of the sand, only the siphon protruding. The eggs, surrounded by the albumen and the secretion which forms the capsules, are poured out of the oviduct into a small cavity in the folded foot. Here the capsule-forming material comes into contact with the sea water and hardens, after which the capsule is released from the foot and another is formed. The average length of time taken by *Sycotypus* in forming a capsule is about three hours, and as the "egg string" may contain as many as seventy capsules, the egg laying may cover a period of several days.

The eggs of *Fulgur* are laid at Beaufort, N. C., during the months of May and June principally.[2] *Sycotypus* lays its eggs at Woods Hole late in August or early in September. The rate of development in these gasteropods is very slow. I can give no definite figures as to the length of the period of development, since the eggs which have been brought into the laboratory do not long continue to develop normally, but from such evidence as I have been able to gather I conclude that the development within the capsules occupies several months; indeed I have frequently found egg strings in midwinter with partially developed embryos, indicating that the eggs which are laid in the fall may not hatch until the following spring. As might be expected where eggs are so greatly laden with yolk, the entire embryonic and larval development is passed within the egg capsules and the young hatch in practically an adult condition.

3. METHODS AND MATERIAL.

The eggs of *Fulgur* and *Sycotypus* are very soft and are difficult to preserve. When removed from the very thick jelly in which they are enclosed within the capsules they usually flatten into thin disks under their own weight, or become otherwise distorted. There are no egg membranes except those formed by the surrounding gelatinous substance, and the thin pellicle of protoplasm which surrounds the egg is too weak to preserve the spherical shape or even to retain the yolk unless the eggs are floating in water or jelly. Since the jelly must in most cases be removed before the fixation of the eggs, special means must be employed to prevent them from bursting or becoming distorted. Many rapid fixing fluids, such as hot sublimate, alcohol and alcohol-acetic mixtures, cause the eggs to crack open, or even to split into fragments after they have been apparently well fixed. After experimenting with many fixing fluids, the only successful method of preservation which I have found, is to open one side of the capsule and place the capsule containing the eggs in the jelly in 20 per cent. formalin for an hour or two, and then shake the eggs out of the jelly into a mixture of 20 per cent. formalin and 70 per cent. alcohol, equal parts, afterwards transferring them to 80 per cent. alcohol. Unfortunately this method leaves shreds of the jelly adhering to the eggs, and as these shreds stain intensely they interfere with the study of the eggs, which are to be stained and mounted entire. To preserve eggs entirely free from the jelly I have found it advisable to slit open the jelly and allow the eggs to drop out into a tall jar of 20 per cent. formalin, or into a mixture of pure formalin (40 per cent.) and a saturated solution of corrosive sublimate, equal parts. If the jar is quite tall (at least eighteen inches) the eggs may be sufficiently hardened before they reach the bottom to preserve their spherical shape, especially if there is a layer of absorbent cotton at the bottom of the jar. After a preliminary hardening in this fluid the eggs may be transferred to other fixing fluids or to alcohol. These eggs were then stained in dilute Delafield's hæmatoxylin, and were mounted entire in the manner described by me (1897, 1902) for other kinds of eggs.

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[2] I wish here to acknowledge my indebtedness to Hon. George M. Bowers, U. S. Commissioner of Fisheries, and to Dr. Caswell Grave, Director of the Fisheries Station at Beaufort, for the many courtesies extended to me while I was at the Beaufort Station.

II. Cleavage.

1. THE UNSEGMENTED EGG.

I have made no attempt to study the phenomena of maturation and fertilization in these eggs since they are particularly unfavorable for such work, owing to the difficulties of fixation, already referred to, and to the great quantity of yolk, which make the eggs difficult to section.

The most striking feature of the unsegmented egg is the extremely small quantity of the nuclear and cytoplasmic material as contrasted with the yolk. The area of cytoplasm and the nuclear spindle shown in Plate XXIII, fig. 1, are but little larger than in the egg of *Crepidula,* though the entire egg of *Fulgur* is about 2,000 times the volume of the egg of *Crepidula.* The great increase in the size of the egg of *Fulgur* as compared with that of *Crepidula* is due almost exclusively to the increased quantity of the yolk.

2. FIRST AND SECOND CLEAVAGES.

It is one of the surprises connected with the development of this egg that although the yolk is so abundant the cleavage is yet holoblastic. When this work was first begun I had thought that the cleavage in this egg, which is larger than the eggs of many cephalopods, might show some resemblances to the meroblastic cleavage of the cephalopod egg. However, this is not the case, for the cleavages, at least as far as the 56-cell stage, are identically like those of *Crepidula* save for the enormous size of the macromeres. In the positions of the mitotic spindles, the direction and rhythm of division, and the shapes, relative sizes and number of the micromeres these two genera are practically identical. In the later development of *Fulgur* the yolk cells do not divide, although their nuclei do, and this may be an approach to the condition found in meroblastic eggs. Many characteristic differences appear between *Fulgur* and *Crepidula* in the later cleavages, and this fact is another evidence in favor of the view, which I have maintained elsewhere (Conklin, 1897, 1898), that the form of the early cleavage is more constant than that of the later cleavage; indeed the early cleavage pattern may be reckoned as one of the most conservative features in the development of any gasteropod.

The first cleavage divides the egg into two equal cells, one of which forms the anterior half of the future animal, the other the posterior half (fig. 2). At the close of this cleavage the nuclei and cytoplasmic areas rotate first in a dexiotropic and then in a læotropic direction, as in *Crepidula*, showing that the first cleavage is spiral in character.

During the first cleavage and again during the second a small lobe is formed at the vegetal pole, which again fuses with one of the macromeres at the close of the cleavage. This is the yolk lobe which has been observed in so many different animals. In forms in which the first two cleavages are equal this lobe is small, *e.g.*, in *Crepidula* it is quite inconspicuous, in *Fulgur* it is larger but still not more than one-sixth the diameter of the macromere; in forms in which the first two cleavages are unequal this lobe may be larger than the macromeres, *e.g.*, *Urosalpinx*. McMurrich mistook this lobe for the polar body, but afterwards (1896) corrected this mistake.

The second cleavage is at right angles to the first and divides the egg into right and left halves. It is not possible to affirm that the median plane of the future animal coincides precisely with the second cleavage plane, but it is evident that the two are very nearly, if not exactly, coincident. In this respect, as also in the læotropic position of the spindles and the relative positions of the daughter nuclei and spheres, this cleavage is similar to the corresponding one of *Crepidula*.

It is a surprising fact that a mitotic figure so small as that shown in fig. 1 can bring about the division of so large a cell. It does not seem possible that the mechanical influence of the amphiaster could cause so large a result, except as it may serve as a stimulus to other forces. It is doubtful too whether cytoplasmic movements of a vortical nature are sufficient to explain the division of a cell body in which there is so small an amount of cytoplasm and so large a quantity of yolk. The superficial layer of cytoplasm is so thin and weak that at first thought it would seem incapable of constricting and ultimately dividing this great cell. But although the factors named seem inadequate to produce so large a result it must be borne in mind that the yolk is relatively soft, almost fluid, and that a considerable quantity of cytoplasm is distributed through the yolk at this stage; furthermore the rate of division is extremely slow, many hours being necessary for the completion of each of the first two cleavages. These are the only equal divisions of the entire yolk which I have seen. Although the four macromeres give rise to many cells in the course of development they still constitute the chief volume of the embryo as late as the veliger stage (pl. XXVIII, fig. 31), thus showing that the yolk content of these cells has remained practically as in the 4-cell stage.

3. SEGREGATION OF THE GERMINAL LAYERS.

The third cleavage is very unequal and separates the first quartet of micromeres from the macromeres in a dexiotropic direction (pl. XXIII, fig. 4); these cells come to lie in the furrows between the macromeres, and their shape is consequently like the corresponding cells in *Crepidula*.

The fourth cleavage (fig. 4) separates the second quartet of micromeres from the macromeres in a læotropic direction. In anticipation of this cleavage the nuclei with the surrounding cytoplasm rotate læotropically until they lie in the upper left-hand corner of each cell, then the mitotic figures appear and the cleavage takes place precisely as in *Crepidula* and other gasteropods.

The first quartet cells then divide læotropically, giving off at their peripheral borders the "turret cells," and a little later the macromeres again divide in a dexiotropic direction, giving rise to the third quartet (fig. 5). Simultaneously the cells of the second quartet divide dexiotropically. These divisions are shown in figs. 5 and 6. As a result of these divisions twenty micromeres are formed, of which eight belong to the first quartet, eight to the second and four to the third (fig. 6). The entire ectoderm comes from these twenty cells. The micromeres are composed almost entirely of cytoplasm and are relatively free from yolk; in the macromeres the cytoplasmic areas surrounding the nuclei lie at the periphery of the cap of micromeres. The visible quantity of the cytoplasm has increased very considerably since the beginning of cleavage, as is readily seen by comparing figs. 1 and 6. This is probably due to the segregation of cytoplasm, originally distributed through the yolk, into the micromeres and cytoplasmic areas of the macromeres, rather than to the conversion of yolk into cytoplasm, since the yolk becomes less fluid and more firm during this period.

In *Fulgur* and *Sycotypus,* as in every other gasteropod so far studied, there are only three quartets of ectomeres. This is especially interesting in view of the enormous size of this egg, where we might expect, if anywhere, to find a much larger number of micromeres formed. McMurrich supposed that a large number of micromeres were budded off from the macromeres in *Fulgur,* and that the number of such micromeres varied with the size of the egg. Viguier (1898) and Fujita (1895) also reached the same conclusion, maintaining that the number of quartets formed in *Tethys* and *Siphonaria* is greater than three. This view is in my opinion wholly without foundation. Whether the egg be large or small three quartets of ectomeres are formed, no more and no less, and by the subdivision of the cells of these quartets all the ectoderm of the embryo is formed. In the cases cited by Viguier and Fujita it can readily be seen, from their own figures, that they have mistaken the subdivision of certain quartet cells for the formation of new quartets from the macromeres.

Since there are only three quartets of micromeres in *Fulgur,* and since these cells must overgrow the enormous yolk, it would seem reasonable to expect that the rate of subdivision of these quartets would be accelerated, as compared with forms in which the yolk is small. However this is not the case; the first quartet divides about the time of the formation of the second quartet, and the latter divides coincidently with the formation of the third quartet, just as in *Crepidula.* In much later stages the micromeres do divide more rapidly, but the early subdivisions of the three quartets is not more rapid in the case of this largest of gasteropod eggs than in the case of the smallest. In every respect except the size of the macromeres, the early cleavage of *Fulgur* is almost identically like that of *Crepidula* or any other prosobranch. In the number, position, shapes and relative sizes of the micromeres and in the positions and sizes of the nuclei and spheres there is the closest similarity between the largest and smallest of gasteropod eggs.

The next division is a highly characteristic one for annelids and mollusks, viz., the formation of the first member of the fourth quartet, 4*d,* by læotropic cleavage of the left posterior macromere.

After its formation it moves over into the furrow between macromeres *D* and *C,* as shown in pl. XXIV, fig. 8. This cell, 4*d,* is formed in advance of the other members of the fourth quartet and it contains a considerable quantity of yolk. In all of these regards it resembles the corresponding cell in all annelids and mollusks, with the exception of the cephalopods, in which the cell-lineage has been studied.

But the feature of particular interest in this cell is its destiny rather than its origin. In all annelids and mollusks, so far as known, the anterior portion of this cell gives rise to most of the mesoderm, while from its posterior portion the terminal part of the alimentary canal arises. This cell is therefore known as the mesentoblast. The history of this cell in *Fulgur* conforms entirely to what we know of it in other forms, as will appear later.

With the formation of the mesentoblast the segregation of the substances of the different germinal layers is nearly completed. I have not observed that the substances composing the mesentoblast differ visibly from those of the ectoblasts and entoblasts, except in the quantity of yolk which these different cells contain. But in *Limnæa*, *Physa* and *Planorbis* I have been able to distinguish the substance of the ectomeres from that of the mesomeres and entomeres before the first cleavage of the egg. In these fresh-water snails, therefore, there is a definite localization of the substances of the different germinal layers, and I have elsewhere shown reason for believing that it is the fact of this localization which determines that the ectoblast shall be separated in three quartets, and that the mesoblast shall arise from one cell of the fourth quartet. Since the segregation of the germinal layers occurs in exactly the same way in *Fulgur* as in these other gasteropods, it seems probable that there is here also the same type of localization of the germinal substances of the egg, and that the enormous accumulation of yolk in this egg has not altered in any fundamental way the localization of its various substances.

The other members of the fourth quartet, 4*A*, 4*B* and 4*C*, are not formed until the 50-cell stage (fig. 9). They are much larger than the cell 4*d* and contain a much greater quantity of yolk. Because of their great size I shall speak of them as the "secondary macromeres." These cells are purely entoblastic in character and give rise to a portion of the alimentary canal. In every one of these details they closely resemble the corresponding cells of *Crepidula*.

4. FURTHER HISTORY OF THE ECTOMERES.

The further history of the three quartets of ectomeres may now be followed through the development up to the time when they give rise to the first recognizable organs. About the time of the formation of the cell 4*d* all of the twenty micromeres divide so that forty micromeres are formed, as shown in fig. 8 (in this figure all the micromeres have divided or are dividing except the two posterior turret cells, which divide a little later, fig. 9). The directions of these divisions and the relative sizes of the daughter cells is the same as in *Crepidula*, and the time at which the divisions occur is also the same, except that the turret cells divide earlier in *Fulgur* than in *Crepidula.*

The latest stage to which I have been able to trace with certainty the lineage of every cell is shown in fig. 9. At this stage there are present the following cells:

Ectomeres	42
Mesentomeres	6
Entomeres	7
Total	55

Fig. 10 shows spindles in two cells of the second quartet, and when the divisions here indicated are completed in the four quadrants the number of cells of known lineage is raised to 59. As a result of this work on the early cleavage it may be affirmed with confidence that in *Fulgur* the lineage of every cell up to about the 60-cell stage is identically like that of *Crepidula.*

a. The First Quartet.—Ectodermal Cross.—In *Crepidula* and many other prosobranchs one of the most striking appearances in the early history of the ectomeres is the formation of the so-called "ectodermal cross." This cross is composed of all the cells of the first quartet, except the turret cells, and of four cells of the second quartet, one of which forms the tip or terminal cell of each arm of the cross. The center of the cross lies exactly at the animal pole, and one of the four arms is anterior, one posterior, one right, and one left. This ectodermal cross, composed of exactly the same cells as in *Crepidula*, is present in *Fulgur* also (fig. 9 *et seq.*). It is not, however, so distinctly marked off from the other micromeres as in *Crepidula*, and it is therefore much more difficult to trace its subsequent history. In pls. XXIV, XXV, figs. 8–16, the outlines of the cross are shown in heavy lines, the center of the cross is marked by a short cross line, and the tip of each arm is lightly stippled. In fig. 8 the cross consists of three cells in each quadrant, an "apical," a "basal" and a "tip" cell (the anterior tip cell is not yet formed). In fig. 9 the basal cells in the right and left arms have divided, and in fig. 10 the basal cell of the anterior arm has also divided. There are at this stage four apical cells and three cells in each arm of the cross, except the posterior one which contains but two. Later, as shown in fig. 11, the basal cell in the posterior arm also divides and at this stage the cross consists of sixteen cells. Still later the apical cells bud off small cells between the bases of the arms, the "rosette" cells of *Nereis* and *Crepidula* (fig. 12). In every one of these details of cleavage the history of the cross in *Fulgur* is like that in *Crepidula*. The latest stage in which I have been able to identify all the cells of the cross is one slightly later than the one shown in fig. 12. At this stage the cross consists of the following cells:

Apicals	4
Rosettes	4
Basals	7
Middles	7
Terminals	4
Total	26

In later stages the entire cross cannot be recognized with certainty. In figs. 13–16 the center of the cross, where the apical cells meet, can be located, and the terminal cells may be seen more or less clearly, but the other cells cannot always be surely identified. In fig. 13 the arms of the cross can be plainly seen, each consisting of four cells in a single linear series, as well as the four derivatives of the turret cells which lie between the arms of the cross, but the apical and rosette cells cannot be identified. In fig. 14 the cross is scarcely apparent at all, and no part of it can be identified with certainty. In fig. 15 a cross is shown with four cells in a series in each arm, just as in fig. 13. Fig. 16 shows a central plate of cells surrounded by heavy lines which probably represents the cross; the center of the apical cells may be recognized, more or less doubtfully, at the place marked by the cross line, and the terminal cells are indicated by the stipples. Midway between the terminal cells on each side of the plate is a group of four cells, also enclosed in heavy lines, which are the derivatives of the turret cells. There are thirty-six cells in the cross in this latest stage in which it is recognizable, eight of which are derived from the second quartet. Deducting these second quartet cells and including the turret cells, we find that there are forty-four cells of the first quartet shown in pl. XXV, fig. 16.

In *Crepidula* seven large cells of the anterior arm of the cross form a peculiar "apical cell plate," while a number of large ciliated cells derived from the posterior arm and the posterior turret cells form a large "posterior cell plate," which becomes the head vesicle of the larva. In *Fulgur* none of these structures are visible at any time, and although the cross may be recognized clearly enough in its earlier stages, it is quite evident that it is not so definite a landmark here as in *Crepidula*, where its cells may be identified with great certainty up to a stage where it consists of sixty-six cells. The turret cells in *Crepidula* grow to an enormous size and remain undivided up to a stage with more than 100 cells; in *Fulgur* they do not become larger than the other cells of the first quartet, and they divide twice before the 100-cell stage, as shown in figs. 13 and 16.

b. Second and Third Quartets of Ectomeres.—As far as I have been able to follow the lineage of these cells in *Fulgur*, it coincides precisely with that of the same quartets in *Crepidula*. The first subdivision of the second quartet in *Fulgur* is shown in fig. 7; the first division of the third quartet and the second of the second quartet in figs. 8, 9 and 10. As a result of the divisions indicated in these figures two cells of the third quartet and four of the second are formed in each quadrant of the egg. By the divisions indicated in fig. 10 one additional second quartet cell is formed in each quadrant, and by the spindles shown in fig. 12 still another cell is formed, making six second quartet cells in each quadrant of the egg shown in fig. 12. All the third quartet cells then divide, as indicated by the spindles in two of the cells in fig. 11, thus giving rise to four third quartet cells in each quadrant. In figs. 12–16 there are six cells of the second quartet and four of the third in each quadrant. Every one of these ten cells has its homologue in the corresponding stages of the egg of *Crepidula*.

This is as far as I have traced the lineage of all the cells of the second and third quartets, though individual cells of these quartets may be recognized in later stages (fig. 17). These cells of the second and third quartets are in general larger than those of the first quartet, which they completely surround. The third quartet cells are especially large, and in some cases (figs. 13, 15, 16) are quite as large as the derivatives of 4*d*.

In the stage shown in fig. 16 there are eighty-eight ectomeres as follows:

First quartet cells	44
Second quartet cells	28
Third quartet cells	16
Total	88

5. FURTHER HISTORY OF THE MESENTOMERES.

The cell 4*d*, formed at the 24-cell stage by læotropic division of the macromere *D*, undergoes dexiotropic cleavage into two equal cells, $4d^1$ and $4d^2$ (fig. 9). At first these two cells lie to the left of the median plane, but they later shift position so that the plane of division between them comes to coincide with the plane of bilateral symmetry. The next division of these cells separates an anterior protoplasmic portion from a posterior yolk-laden one (fig. 9), and this process is repeated at a slightly later stage (fig. 12), thus giving rise to six cells derived from the cell 4*d*, three on each side of the median plane.

Of these cells the two anterior ones on each side are small protoplasmic cells which lie wholly under the layer of ectodermal cells; these cells give rise to the mesoblastic band of each side. The posterior cells are much larger and contain a considerable quantity of yolk; they are only partially covered by the cap of ectodermal cells (fig. 12); they ultimately give rise to the terminal portion of the intestine. I have not followed in detail the cell lineage of these cells in later stages, owing in part to the fact that they lie close under the thin layer of ectoderm, and it is frequently difficult to distinguish one from the other in surface views. However it is sufficiently evident that these cells show fundamental resemblances in origin, history and destiny to the mesentoblast cells of *Crepidula* and other gasteropods. The "mesoblastic bands" of *Fulgur* do not present a single series of cells, as in some annelids and mollusks, but rather a broad, irregular band of cells which has the general form of the letter Y. The stem of the Y lies in the median plane behind the area of the shell gland, while its two branches diverge on each side of this structure (figs. 19–24).

Some distance in front of the anterior ends of these bands a few cells appear beneath the ectoderm, which are probably the homologues of the "larval mesoderm" of other forms (figs. 19–22). They are in close relation to the ectoderm cells which give rise to the cerebral ganglia. I have not followed these "larval mesoderm" cells until they give rise to mesodermal structures, but have classed them as mesoderm because they lie beneath the surface and resemble in appearance the cells of the mesodermal bands.

6. THE ENTOMERES.

Up to a stage when there are approximately fifty cells in the entire embryo the entomeres consist of the four macromeres only. These macromeres are all of about the same size, and they contain an enormous quantity of yolk as compared with the amount of protoplasm. Each macromere contains an area of cytoplasm relatively free from yolk, in which lie the nucleus and sphere. These cytoplasmic areas lie as near as possible to the animal pole and to the free surface of the cell; with the extension of the cap of ectomeres these cytoplasmic areas with their enclosed nuclei and spheres are not overgrown by the ectomeres, but they move out over the yolk at the margin of the ectodermal cap, and this position they retain until the blastoderm has overgrown much of the yolk (pl. XXVII, fig. 26).

At a stage of about fifty cells, the macromeres *A*, *B*, and *C* divide in a læotropic direction, giving rise to the remaining members of the fourth quartet, 4*A*, 4*B* and 4*C* (fig. 9). These cells are very large and full of yolk, and for these reasons I have called them "secondary macromeres." They come to lie in the furrows between the macromeres, and in this position they are partly overgrown by the advancing ectoderm and partly shoved before it. They serve as an excellent means of orienting the egg and embryo, since they are found at the right, left and anterior poles only, the posterior pole being occupied by the derivatives of the much smaller mesentoblast cell, 4*d*.

The secondary macromeres are purely endodermal in character. Although their nuclei subdivide (pls. XXV, figs. 17, 18, *et seq.*), their cell bodies usually remain undivided until the closure of the blastopore (pl. XXVIII, fig. 31). Throughout all of this period the entomeres consist of the four macromeres and of these three secondary macromeres. Even as late as the gastrula stage, shown in fig. 31, these are the only entoderm cells present except those derived from the cell 4*d*, which give rise to the terminal portion of the intestine (*In.*, figs. 29–31).

In *Crepidula* a fifth quartet of cells, which are purely entodermal in character, is formed from the macromeres about the time of the closure of the blastopore; and at the same time the cells 4*A*, 4*B* and 4*C* divide. These divisions have not been observed in *Fulgur*, but, with these exceptions, the origin, history and destiny of the entomeres of *Fulgur* are almost precisely like those of *Crepidula*. The four primary macromeres are similar in all regards, save only in bulk; the secondary macromeres arise at the same cell stage and behave in the same manner in these two genera. In *Crepidula*, as in *Fulgur*, these seven cells, together with the entoblastic derivatives of the cell 4*d*, constitute for a long period the whole of the entoblast.

With the stage represented by fig. 17, in which there are about 121 cells, the ectomeres become so numerous that their lineage cannot readily be traced. At a slightly later stage, regions of the blastoderm begin to differentiate into characteristic structures. We shall therefore consider this stage as the close of the cleavage and the beginning of the period of organ formation.

In conclusion we find that the cleavage of *Fulgur* resembles that of *Crepidula* in almost every detail. Such slight differences as do exist, as, for example, in the relative development of the "cross" in the two forms, are only such as frequently exist between other genera in which the eggs are about equal in size. In short the enormous size of the *Fulgur* egg, which has so greatly altered the relative proportions of cytoplasm and yolk, and of micromeres and macromeres, has not modified in the least the pattern of the cleavage. It has not modified the localization in the egg and the segregation in the cleavage of the substances of the germinal layers. In all of these respects the egg of *Fulgur* is typically like that of other gasteropods, and it shows no approach whatever to the condition found in the eggs of cephalopods.

7. RELATIVE SIZES OF NUCLEI AND CELLS.

The great quantity of yolk in the egg of *Fulgur* introduces some interesting cytological conditions. I have already called attention (p. 325) to the extremely small nuclear spindle in the first cleavage, and to the problem of how this little spindle and the small amount of cytoplasm around it can bring about the division of so great a cell body. Another, though related, problem is found in the relative sizes of the nucleus and cell body at different periods of the cleavage.

In all the early cleavages the nuclei and spindles are very small, even in the macromeres (figs. 1–6). Indeed the nuclei and spindles in the macromeres are not larger than those in the micromeres during the formation of the three quartets. After the formation of the third quartet (fig. 7) there is a relatively long resting period for all of the macromeres except *D*, consequently the nuclei in *A*, *B*, and *C*, fig. 8, have grown to nearly double the diameter of those in fig. 7. The macromere *D* divides before its nucleus becomes very large, giving rise to the mesentomere, 4*d*. About the 50-cell stage, fig. 9, the macromeres *A*, *B*, and *C* divide, giving rise to the secondary macromeres 4*A*, 4*B*, and 4*C*. The mitotic figures for this division are quite large and are proportional in size to the resting nuclei from which they arise.

From this stage onward the nuclei of all the macromeres enjoy a long "rest," during which they grow to double their maximum diameter at any previous stage (figs. 9–24). The greatest diameter of the nucleus during the 2-cell and 4-cell stages is about 40 μ; during the formation of the three quartets the nuclear diameter is not greater than this, but rather smaller; before the formation of the secondary macromeres (fig. 8) the diameter of the nuclei in *A*, *B*, and *C* is about 50 μ; at the close of their long rest and just before their next division (fig. 24) the nuclei are about 80 μ in diameter.

The diameter of the unsegmented egg is about 1,700 μ; that of each of the macromeres during the formation of the quartets about 970 μ; while in the stages after the formation of the fourth quartet it is somewhat less, say about 900 μ. Therefore, although the diameter of the cell body in these later stages is but little more than half that of the unsegmented egg, the nuclear diameter is twice as great as at any previous stage.

The ratio of the nuclear diameter to the cell diameter, the "Kernplasmarelation" (k/p) of Hertwig (1903), is about 1 : 40 in the unsegmented egg; about 1 : 24 in the 4-cell to 24-cell stages; about 1 : 20 just before the formation of the secondary macromeres; and about 1 : 12 at the close of the long resting period of the macromeres, shown in fig. 24.

In the micromeres of the 8–24-cell stages the ratio of the nuclear diameter to the cell diameter is about 1 : 5; and in the later stages, such as fig. 18, it rises to 1 : 3.

It seems perfectly evident from these figures that the absolute size of the nucleus is dependent not only upon the size of the cell, but also upon the length of the resting period; and by the same showing the length of the resting period is not determined primarily by the size of the nucleus. In all the earlier divisions of the macromeres of *Fulgur* the division comes on when the nucleus is relatively small (k/p = 1 : 24); in later stages it does not come on until the nucleus has grown to twice this size (k/p = 1 : 12).

In *Crepidula* I found (1902) that the size of the nucleus, chromosomes, centrosomes, spindles and asters was, in the last analysis, proportional to the volume of the cytoplasm, and I tried to show that the immediate cause of division was the growth of the nucleus to a point where the ratio of nucleus to cytoplasm exceeded a critical limit. This is certainly not the principal factor which brings on division in *Fulgur*; and the fact that mitosis may occur in one cleavage when the nucleus is relatively small and in another not until it becomes very large, suggests that the moment of division may be dependent upon some intrinsic condition in the nucleus or centrosome, rather than upon the ratio of nuclear size to cell size. Support is lent to this view by the phenomena of oogenesis; here we have in the germinal vesicle the largest nucleus in the entire life cycle, following upon one of the longest resting periods, while the second maturation division follows immediately after the first; here also the moment of division seems to depend upon intrinsic conditions in the cell.

In the division of the cell body *Fulgur* presents some interesting conditions. Attention has been called to the fact that although the spindle in the first cleavage is very small the entire egg divides. Equally surprising is the fact that in the divisions of the primary and secondary macromeres subsequent to the formation of the fourth quartet, the nucleus only divides while the cell body remains undivided (figs. 17–28). The cell bodies of these entomeres do not divide again until very late in development, if at all; even in figs. 34 and 35, in which the embryo shows many definitive structures, the furrow between two of the original macromeres can be seen running obliquely through the region below the head vesicle (*H. V.*).

It seems to me that the following explanation may be offered of this apparent anomaly. In the early stages of development the cell substance is quite fluid, as has been pointed out (p. 326), whereas in the later stages the substance of the macromeres becomes much more consistent. In the later stages of development the macromeres do not show the tendency to burst or to undergo distortion during fixation, which is so troublesome in the earlier stages; this is, I believe, due to the greater consistency of the yolk during the later stages. This same difference in consistency may explain also the curious fact that the entire cell divides in the early stages, whereas only the nucleus divides in later stages.

In this connection attention may be called to the fact that there is apparently a great increase in the quantity of cytoplasm in the later stages, as compared with the earlier ones (cf. figs. 1, 6, 25). This increased quantity of cytoplasm might be due to the liquefaction of the yolk and its transformation into cytoplasm, or it might be the result of the segregation into the ectodermal cap of cytoplasm originally spread through the yolk substance. It is probable that both of these processes occur, but in view of the increasing consistency of the yolk during the period of quartet formation, it seems very probable that most of the cytoplasm of the early stages was present as such, but in a diffused form, in the unsegmented egg.

III. Organogeny.

It has been shown that there are but few and minor differences between *Fulgur* and *Crepidula* in the cleavage up to a stage of about 100 cells (fig. 16). After this stage the differences in development become more marked. The first notable difference is found in the number of micromeres; by repeated divisions of the three quartets of ectomeres these cells become very numerous in *Fulgur,* as shown in figs. 17, 18, *et seq.*, whereas in *Crepidula* the number remains relatively small. It is evident that the same cell stages in the two no longer represent similar stages in differentiation; for example, the shell gland is one of the first organs to appear, and is first visible in *Crepidula* when there are about 250 cells present (Conklin, 1897, fig. 74); while it first becomes visible in *Fulgur* when there are about 1,000 cells (fig. 23). The period of the closure of the blastopore is very different in the two; in *Crepidula* this occurs before any organs are visible (fig. 65), in *Fulgur* only after almost all the larval and many of the definitive organs are present (fig. 31).

Other striking differences between *Fulgur* and *Crepidula* are found in the form and character of the different organs and in the time at which they appear, but most of all in the method of formation of these organs and in their relation to the yolk.

1. OVERGROWTH OF THE YOLK AND CONCRESCENCE OF THE EMBRYO.

Owing to the fact that the entomeres are so large in *Fulgur,* organs begin to differentiate long before the closure of the blastopore, indeed while the blastoderm is still a small cap at the animal pole of the egg (figs. 23–26). The organ bases are here spread out as in a mercator's chart, whereas in *Crepidula* the organ bases do not appear until the blastoderm has overgrown the yolk and they occur over the sphere, so that only a few of them can be seen from a single point of view.

In the earlier stages of *Fulgur* the organ bases occur in the anterior half as well as in the posterior portion of the blastoderm (figs. 21–23); later by the very rapid growth of the median anterior portion of the blastoderm these organ bases are displaced laterally and posteriorly until they come to form a kind of germ ring, or crescent, along the posterior margin of the blastoderm (figs. 24–28). This germ ring contains the bases of all the future organs, whereas the rest of the blastoderm forms a kind of yolk sac.

The manner of the overgrowth is represented diagrammatically in the accompanying text figure. The outlines of the blastoderm in successive stages of the overgrowth are indicated by the numerals 1–8. By superimposing on the same figure these different stages of the overgrowth it is possible to see at a glance the character and relative amount of the movement of the different organ bases. Thus in 1, the center of the blastoderm is approximately the center of the animal pole; in front of this lie the cerebral ganglia (*cb*) and velar row (*v*), while the buccal ganglia (*bc*) lie at the right and left margins of the blastoderm. Posterior to the animal pole is

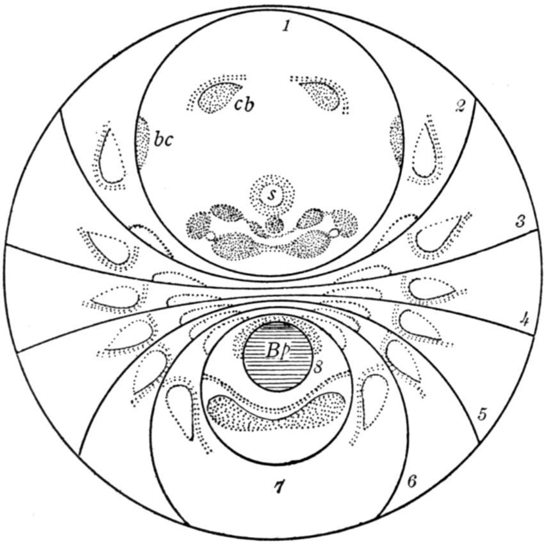

DIAGRAM illustrating the more important phases in the overgrowth of the yolk by the blastoderm in *Fulgur carica*. The outer circle represents the outline of the entire egg, and the smaller circles, numbered 1 to 8, represent the margins of the blastoderm in successive stages of the overgrowth. The egg is represented as seen from the posterior pole, and in order to bring all stages into one figure the arc through which the posterior margin of the blastoderm moves is represented as less than is actually the case. The outline of the blastoderm and the positions of the organ primordia in stage 1 correspond to figs. 25 and 26, plate XXVII; stages 2 and 3 correspond to fig. 27; stage 4 to fig. 28; stage 5 to fig. 29; stages 6 and 7 to fig. 30; stage 8 to fig. 31. In stage 1 the velar cells are represented as a double line of stipples anterior and lateral to the cerebral ganglia (*cb*); the buccal ganglia (*bc*) lie at the lateral margins of the blastoderm; immediately posterior to the shell gland (*s*) is the primordium of the intestine; on the left of this is the abdominal ganglion, and on each side of the latter are the parietal ganglia, the pleural ganglia, the otocysts and the pedal ganglia. In stages 2–7 the cerebral and buccal ganglia are represented in dotted outlines; in stages 1 and 8 they are stippled, as are the other primordia.

found an organ complex which includes the shell gland, intestine, abdominal, parietal, pleural and pedal ganglia, and the otocysts.

By the growth of the median anterior portion of the blastoderm, the cerebral ganglia and velar cells and the buccal ganglia are carried around to the successive positions indicated in the diagram, while the organ complex described above undergoes little movement. The diagram is faulty in that it shows too little movement of the organ complex of the posterior lip, and thus represents the final position of the blastopore too near the posterior pole, but it represents fairly well the relative movements of the different organ bases during the first four stages shown (1–4). Reference to the first and second cleavage furrows between the macromeres, shown in figs. 25–28, will show that the posterior margin of the blastoderm remains relatively fixed during this period, while at the same time the blastoderm extends forward and laterally; still later the blastoderm extends posteriorly also, and finally the blastopore closes at the vegetal pole (fig. 30).

2. APICAL INVAGINATION.

The first structure which appears in the course of development is an invagination of ectodermal cells just anterior to the animal pole and in the region of the anterior arm of the cross. This invagination is preceded by a broad depression of the blastoderm (fig. 18), and then the center of this depression becomes deeply cup-shaped (fig. 19), and finally tubular in form (fig. 20). The axis of this tubular invagination at first runs forward under the ectoderm; then it becomes perpendicular to the surface of the blastoderm; and subsequently it runs backward under the ectoderm, its opening being at its anterior end (fig. 20). The inner, blind end of this invagination lies near the anterior end of the polar furrow, and in some instances, though not in all, a few cells are separated from the invagination at this point. The anlagen of the cerebral ganglia are formed on the right and left of this invagination, but they do not appear to be derived from it. Similarly a few mesoderm cells, which probably correspond to the "larval mesoderm" of other mollusks, lie on each side of the invagination, though they are not derived from it (see the black nuclei in figs. 19 *et seq.*).

Subsequently this conspicuous and definite structure completely flattens out and disappears (figs. 21 *et seq.*), leaving not a trace behind. This fact seems so remarkable and the significance of the invagination is so problematical that I have devoted considerable time and effort to the study of it.

It is evident that this invagination is not merely the result of the sinking in of the blastoderm over a cavity in the yolk. There is frequently a cavity between the macromeres, but this invagination invariably lies anterior to this cavity, and in many cases it actually forces its way into the substance of the yolk. In some cases also a prominent evagination is formed instead of an invagination, showing that there is very active growth in this region of the blastoderm.

It seems to me probable that this invagination is a mechanical adaptation to secure a rapid extension of the anterior half of the blastoderm over the yolk. During the growth of the invagination the area of the blastoderm remains stationary, or actually grows smaller; as soon as it flattens out there is a very rapid increase in the area of the blastoderm (cf. figs. 20 and 21), especially of that portion lying anterior to the first cleavage plane. Coincidently with this increase in area the secondary macromere, *4B*, begins to move forward in the furrow between the macromeres and the blastoderm rapidly extends forward. The remarkable extent of the growth of this anterior portion of the blastoderm may be seen by comparing the positions of the organ bases in figs. 19–30. In the earlier figures the shell gland and the two cerebral ganglia form the angles of an isosceles triangle, the base of which is directed forward (figs. 21 and 22); in later stages the ganglia separate more widely and the triangle becomes equilateral (figs. 23 and 24); still later the cerebral ganglia are separated so widely that they lie in line with the shell gland (figs. 27 and 28); and finally the cerebral ganglia again approach each other on the ventral side of the embryo (figs. 29 and 30). The greatest growth of the blastoderm takes place in the area between and in front of the cerebral ganglia, in the very region of the apical invagination, and it seems reasonable to suppose that the remarkable growth of this region is associated with the formation and subsequent flattening out of this structure.

McMurrich (1886) described at some length this invagination, and he compared it with a similar formation observed by Blochmann (1883) in *Neritina*, and by Sarasin (1882) in *Bythinia*. He says: "It seems very strange that an invagination so well marked as it is in *Neritina* and *Fulgur* should disappear and leave no trace of its existence, but so it seems to do." His further conclusion that it would be found to occur in most, if not all, of the prosobranch gasteropods has not been justified by later studies.

3. SHELL GLAND, SHELL AND MANTLE.

The shell gland is one of the earliest and largest of the developing organs, and is the one most instrumental in shaping the form of the embryo. It appears as an aggregation of ectodermal cells in the median plane posterior to the apical pole (figs. 21, 22). These cells, which are probably derived from the cell *2d*, increase greatly in number and form a saucer-shaped depression (figs. 23–26, *Sh.*). This depression then becomes deeper and smaller in surface area (figs. 27, 28) and subsequently it evaginates in the manner characteristic of gasteropods, the margin of the gland forming a ridge, the mantle edge, while the center is covered by the cuticular substance which forms the earliest shell; beneath this shell is a very thin layer of ectoderm, in which the nuclei are few and far apart. The margin of the shell gland, or mantle edge, is at first circular in outline; then it becomes uniformly elliptical (figs. 29 and 30); finally it continues to extend in all directions, except where it comes into contact with the organ complex on the ventral side of the embryo; here the growth of the mantle edge is arrested, and consequently a notch in the developing shell and mantle appears here, which notch grows deeper as the mantle edge extends farther (figs. 31–36).

The area covered by the shell grows larger continually and the yolk appears to slip through the ring formed by the mantle edge, while at the same time the blastoderm and its organ anlagen are retained in front of this ring. In this way the yolk slips out of the anterior portion of the embryo, and the tension on the blastoderm being relieved in this manner, the latter is free to undergo the foldings necessary to form the head vesicle, velum, larval kidney and heart; at the same time the mantle edge, continuing to grow forward on the dorsal side, gives rise to the mantle fold and mantle chamber. On the ventral side the forward growth of the mantle edge is stopped by the organ complex (figs. 35, 36).

In the earlier stages of this overgrowth, the mantle edge and organ complex are apparently bilaterally symmetrical; in later stages it can be seen that the organs are more developed on the right side (left in ventral view) than on the left, and consequently the notch in the mantle edge is displaced from the median plane toward the right. In this way the assymetry of this gasteropod makes its appearance. Along the left side of the notch (right in ventral view) a cellular thickening of the blastoderm occurs near the mantle edge (figs. 34, 35, *CmM.*) which gives rise to the columellar muscle, while the shell formed along this portion of the mantle edge is the columella.

It is well known that in annelids the ectoderm of the trunk is derived from the ectomere 2*d* (= X). In *Fulgur, Crepidula* and several other gasteropods, cells derived from the ectomere 2*d* give rise to the mantle edge and fold and to the layer of cells covering the yolk under the shell. This fact suggests that the elongation of the embryo through the ring of the mantle edge may possibly be comparable to the elongation of the trunk of the annelid.

4. NERVOUS SYSTEM AND SENSE ORGANS.

In their earliest stages the organ bases may be recognized by the fact that the nuclei are closer together and the protoplasm stains more deeply than in other portions of the blastoderm. In the case of the nervous system certain of the ganglia are from the first clearly distinguishable, whereas some others in the organ complex posterior to the shell gland (fig. 23) cannot be distinguished until a later stage.

a. Cerebral Ganglia.—The group of cells which will form the cerebral ganglia appears at a very early stage. It is shown in figs. 19 and 20 on each side of the apical invagination, and closely connected with the group of cells lying beneath the superficial layer, which I have identified, somewhat doubtfully, with the "larval mesoderm" (fig. 21, *Lm.*). The cells which give rise to the cerebral ganglia arise in the region of the blastoderm, on each side of the anterior arm of the cross and in front of the transverse arms, probably from the "rosette" cells, exactly as in *Crepidula,* and they probably correspond in origin as well as destiny to the cells of the "cephalic neural plate" of *Nereis* (cf. Conklin, 1897, p. 110).

With the great growth of the anterior part of the blastoderm, which follows the flattening out of the apical invagination, these cerebral ganglia are carried laterally until they lie near the margin of the blastoderm (figs. 21, 22, *Cb.*). In figs. 23 and 24 they lie still nearer the margin, and their protoplasm and nuclei are somewhat more condensed than in the previous stage; the bases of all the organs now stain more deeply and they are all connected together by deeply staining protoplasm (figs. 23, 24). In all these stages the cerebral ganglia lie anterior to the middle of the blastoderm and are the anteriormost organs present. Later they are carried back until they lie in the posterior lip of the blastopore and at opposite ends of a transverse line which lies nearly in the plane of the first cleavage, and along which line most of the organ bases of the embryo are found (text fig. and figs. 27, 28). Still later, by a continuation of the movement already described, the cerebral ganglia are carried posterior to the first cleavage plane and to the other organs of the posterior lip, and they then approach each other on the ventral side of the embryo, and come to lie on each side of the blastopore (figs. 29, 30). This movement of the cerebral ganglia may be better described by pointing out their position with reference to the shell gland, which may be considered as a fixed point. In earlier stages the shell gland forms the apex of an isosceles triangle, the other angles being formed by the cerebral ganglia; then by the separation of the ganglia the triangle becomes equilateral, and still later the angle at the apex increases until the ganglia and the shell gland lie in one straight line; then by a continuance of the movement of the ganglia a triangle is formed on the opposite (ventral) side of the shell gland, which develops in the inverse order of that on the dorsal side (text figure). In the stages shown in figs. 30 and 31 the cerebral ganglia lie lateral and slightly posterior to the blastopore; in fig. 32 they lie on the sides of the blastopore, and a process is growing out from each toward the other in front of the blastopore; in fig. 33–36 they lie in front of the blastopore and are closely connected together by the cerebral commissure.

Along the posterior edge of each cerebral ganglion a leaf-like process is formed which is the tentacle (figs. 35, 36, *T.*), and at the outer end of the furrow which separates the tentacle from the blastoderm the eye appears (figs. 34–36).

b. Buccal Ganglia.—Lateral and slightly posterior to each cerebral anlage is a group of cells which gives rise to the buccal ganglion (figs. 22, 23, *Bc.*). These cells lie at the very periphery of the blastoderm and are probably derived from the ectomeres 2*a* and 2*c*. In the overgrowth of the yolk they accompany the cerebral ganglia, moving from the dorsal to the ventral side in the posterior lip of the blastopore (figs. 27–29), and finally coming to lie along the posterior border of the blastopore (fig. 30). When the blastopore closes the buccal ganglia of the two sides come into contact on the posterior side of the blastopore (fig. 31 and text figure).

c. Pleural, Pedal, Parietal and Abdominal Ganglia.—The other ganglia of the nervous system form part of the complex of organs lying between the shell gland and the posterior lip of the blastopore. The pleural ganglia lie on each side of the shell gland and on the lateral borders of the organ complex (figs. 23, 24); the pedal ganglia lie on the median side of the pleurals; close behind the shell gland are the groups of cells which give rise to the parietal and abdominal ganglia. The anlagen of these ganglia are not clearly separated at this time, and only in the later stages (fig. 28 *et seq.*) are they quite distinct. When the foot begins to appear (fig. 31) the pedal ganglia are included within it, and the pleural ganglia lie on each side of the foot, while the otocysts lie between the two (fig. 28 *et seq.*). The otocysts are here, as elsewhere, formed as an invagination of the superficial ectoderm. All the ectodermal organs of this region are derived from the cell 2*d*, with the possible exception of the pleural ganglia, which lie on the lateral borders of the organ complex and may therefore come, in part, from the cells 3*c* and 3*d*. In annelids the ectoderm of the trunk region and the ventral neural plate, which gives rise to all the nervous system posterior to the mouth, come from this same cell, 2*d*, the "first somatoblast." Lillie (1895) found the same condition among lamellibranchs.

Among the gasteropods this cell is not larger than the other members of the second quartet, but it is destined to give rise to all the important ectodermal organs posterior to the mouth. In this fact we have a striking illustration not only of the value of cell-lineage in comparative embryology, but also of the fundamental similarity of annelids, lamellibranchs and gasteropods.

The parietal and abdominal ganglia, with their connectives, are recognizable as thickenings of the blastoderm in a stage as early as fig. 28, but they are not clearly distinguishable until a later stage (figs. 30, 33, 34). In these figures the entire nervous system is easily recognizable. The cerebral ganglia are connected by the cerebral commissure anterior to the mouth, and they are united with the pleurals and pedals by the cerebro-pleural and the cerebro-pedal commissures respectively on each side of the œsophagus. The parietal ganglion of the left side lies almost directly behind the foot, and is connected with the pleural of the left side by the pleuro-parietal connective; the parietal of the right side lies on the right side of the foot and is connected with the pleural of that side, while the two parietals are connected together by the nerve loop which runs alongside of the intestine and near its middle bears the abdominal, or visceral, ganglion.

In these and the following stages (pl. XXVIII, figs. 35 and 36) the entire nervous system and the twisting of the parietal loop may be seen with diagrammatic clearness. This twisting, which is a part of the general movement toward the right of all the organs posterior to the foot, carries the left parietal ganglion to the right side of the foot, where it lies in contact with the right pleural, while the abdominal lies still farther toward the right and the dorsal side (fig. 36); at the same time the right parietal is carried up to the dorsal midline, where it lies on the dorsal side of the œsophagus (fig. 36). The entire nervous system, except the buccal ganglia, which are here hidden from view by the cerebrals, is shown in very nearly its definitive position in fig. 36. All of the six pairs of ganglia may be recognized in fig. 30, and four pairs are recognizable in fig. 23.

In all of these regards the nervous system of *Fulgur* shows fundamental resemblances to that of *Crepidula*. To one who had not observed the earlier stages in the origin of the cerebral ganglia it might seem that these ganglia, which lie so near the periphery of the blastoderm and so closely connected with the other organs of the posterior lip, could not possibly have arisen from cells of the first quartet anterior to the transverse arms of the cross. It is only by a study of the early history of these ganglia and their subsequent movements that one can determine that the cerebral ganglia, and indeed all the ganglia, arise in corresponding parts of the egg in *Fulgur* and *Crepidula*. Such a study shows that amidst all the differences in the mode of development of these ganglia, there is the most fundamental resemblance in their places of origin in the egg, and that therefore the localization of the germinal substances of the egg in the early cleavage cells follows the same pattern in these two genera.

5. THE VELUM.

The velum arises in close relation to the cerebral ganglia, and its place of origin furnishes another striking illustration of what are apparently fundamental differences between *Fulgur* and *Crepidula*, but which are in reality fundamental resemblances. In *Fulgur* the velum is first visible as a dense band of nuclei on the median and posterior sides of the cerebral ganglia (fig. 28, *V.*). These velar bands are widely separated from one another and are entirely disconnected. In later stages these velar bands more completely encircle the cerebral ganglia; each has somewhat the form of a parabola, one limb of which runs forward in the lip of the blastopore, while the other, which lies posterior to the cerebral ganglion, runs out towards the lateral regions of the embryo and there ends in the general blastoderm (figs. 29, 30). Subsequently, when the blastopore narrows and closes, the two limbs which run forward in its lips unite in front of the mouth (figs. 31, 32), while the lateral limb turns forward over the sides of that portion of the embryo which will become the head vesicle (figs. 33, 34). From the posterior side of each band there is given off a branch which runs across the embryo posterior to the mouth and there joins its fellow of the opposite side, thus giving rise to the post-oral band, while that portion of the velum which runs in front of the mouth is the pre-oral band. Both of these bands were observed and described by McMurrich (1886).

In subsequent stages the velum is drawn out into a prominent bilaminar fold (figs. 35, 36). This velar fold or lobe grows out to a very great size, much larger than is shown in fig. 36, the ciliated velar cells being borne around its edge, as in other prosobranchs. By the beating of these cilia it is probable that the embryos are able to move very slowly within the egg capsules, though they never swim freely, the young escaping from the capsules only after the velar lobe has been absorbed. The velum in *Fulgur* is not a highly sensitive and contractile organ, as in *Crepidula* and in many other forms; apparently, it cannot be retracted, even in its most fully developed stage, and it is needless to say that in the stage shown in fig. 36 there is no cavity into which the velum, the head or the foot could be retracted.

From the preceding account it is evident that during the later stages of its development the velum in *Fulgur* is much the same as in *Crepidula* or any other prosobranch. In its early history, however, it seems to be altogether different. It appears, as has been said, on the median and posterior side of the cerebral ganglia when these structures lie far apart in the posterior margin of the blastoderm (fig. 28); in *Crepidula* it first appears about the time of the closure of the blastopore as a transverse row of cells in front of the mouth. In *Fulgur* it consists of two bands of cells on opposite sides of the embryo, which are entirely separate from each other; in *Crepidula* it is a single structure continuous from side to side.

However, in spite of these striking differences, it can be shown, I think, that the velum has a similar origin in both of these genera. Traces of the velum in *Fulgur* may be found at a much earlier stage than that shown in fig. 28; thus in fig. 23 a row of what I take to be velar cells may be seen on the lateral and anterior sides of the cerebral ganglia (the nuclei of these cells are shown in heavy outline). The position of this row of cells indicates that it has arisen from the cells adjoining the ganglion on the anterior and lateral sides, and since the ganglia themselves come, in all probability, from the "rosette" cells, the velar cells must have come from the anterior "turret" cells and perhaps also from the terminal cells of the transverse arms of the cross. In short, the velar cells arise in *Fulgur* in the same region and probably from the same cells as in *Crepidula.* Here again, as in the case of the cerebral ganglia, there is fundamental agreement between *Fulgur* and *Crepidula* in the early and late stages in the development of the velum; it is only in those stages of the overgrowth of the yolk, which are undoubtedly highly modified in *Fulgur*, that we find striking differences between these two genera.

6. BLASTOPORE, MOUTH AND ŒSOPHAGUS.

By the very great growth of the blastoderm in the region of the apical invagination the anterior portion of the blastoderm rapidly surrounds the yolk, while its posterior margin, containing all the organ bases, remains relatively fixed in position near the animal pole (figs. 25–28). Subsequently this posterior margin also extends over the yolk to such an extent that the blastopore finally lies at the vegetal pole of the egg (fig. 30). The anterior lip of the blastopore is always composed of an extremely thin layer of cells, and this portion of the blastopore is usually circular in outline; the posterior lip of the blastopore is always composed of a thicker layer of cells than the anterior lip, and it is not circular but is compressed laterally, as shown in fig. 30.

The blastopore then narrows until it is almost if not entirely closed (figs. 31, 32), and an invagination of ectoderm cells occurs here, which is the stomodæum. The mouth is formed at the very place where the blastopore closes, and the œsophagus is formed by the elongation of the stomodeal invagination (fig. 36, *Œ*.).

In the closure of the blastopore and the formation of the mouth and œsophagus *Fulgur* completely resembles *Crepidula.*

7. INTESTINE AND OTHER PORTIONS OF THE ALIMENTARY CANAL.

The intestine is formed from enteroblast cells derived from the mesentomere 4*d*. These enteroblasts lie in the midline behind the shell gland and they constitute the stem of a Y-shaped group of cells, the branches of the Y being the mesodermal bands (figs. 22, 23). In its earliest stages the intestine is a round, densely staining group of cells, which lies at a lower level than surrounding portions of the blastoderm. After the posterior margin of the blastoderm has extended over to the ventral side of the embryo, the intestine lies between the margin of the shell gland behind and the visceral nerve loop and abdominal ganglion in front (figs. 28–30).

About the time of the closure of the blastopore the anlage of the intestine elongates toward the right (left in ventral view), becoming at first elliptical in shape (fig. 31) and then tubular (fig. 32 *et seq.*). Up to the time of this elongation it lies in the median plane; after this it, together with other organs posterior to the foot, moves to the right. The end of the intestine which remains nearest the midline is the anal end, though the anus does not form until a much later period; the end farthest to the right is the gastral end. The intestine lies just in front of and parallel with the edge of the shell; in fig. 32 its course is nearly transverse to the long axis of the embryo, the notch in the shell being at this stage very shallow. As the shell grows forward on the dorsal side more rapidly than on the ventral, this notch grows deeper, and the right edge of the shell becomes parallel with the long axis of the embryo (figs. 33–35); at the same time the intestine turns from a transverse to a longitudinal course. The visceral nerve loop accompanies the intestine in this movement, the abdominal ganglion always lying close to the mesial side of the intestine.

Finally the mantle cavity begins to form on the mesial side of the intestine, and the latter is thus carried into the roof of the mantle chamber, the anal end of the intestine remaining near the mantle edge, while the gastral end reaches to the deepest portion of the mantle chamber (fig. 36). In all of these details as to the formation of the intestine there is striking similarity between *Fulgur* and *Crepidula.*

In *Crepidula* the gastral end of the intestine opens into a lumen between the yolk cells, which give rise to the stomach and liver. In *Fulgur* I have observed no such lumen between the yolk cells, and while I think it probable that these cells form the stomach and liver in this animal, I have not traced their history far enough to speak with certainty upon this point. In *Fulgur* as in *Crepidula* the secondary macromeres lie at the inner end of the stomodæum (fig. 31), and they probably form that section of the alimentary canal immediately following the stomodæum.

8. THE FOOT.

The development of the foot is practically the same in *Fulgur* as in other prosobranchs. It appears as a thickening of that portion of the blastoderm lying between the otocysts behind, the blastopore in front, and the pedal ganglia on the sides (figs. 29 and 30). It is at first wide in transverse direction and narrow antero-posteriorly and it is bilobed, the two lobes being separated on the side of the blastopore by a groove in which the blastoderm remains thin (figs. 30 *et seq.*). The foot then gradually rises above the general level of the blastoderm until it becomes prominent, becoming about half as wide from side to side as in earlier stages (figs. 33, 34); at the same time it takes into itself the pedal ganglia and otocysts.

At the posterior end of the groove between the two lobes an invagination is formed which becomes the pedal gland (*P. G.*, figs. 33, 34). A transverse furrow on the surface of the foot then constricts off a smaller anterior lobe, the propodium, from a larger posterior one, the mesopodium and metapodium (figs. 35, 36).

9. LARVAL AND DEFINITIVE KIDNEYS.

Running laterally from the foot a ridge of cells develops on the right and left sides; this ridge lies some distance posterior to the velum, with which it is nearly parallel; its free border, or crest, becomes crenated (figs. 34, 35, *Ex.K.*). This is the larval or external kidney and, as in other prosobranchs, consists of large ectodermal cells which become loaded with nitrogenous waste substances. It is an interesting fact that the larval kidney of *Fulgur* does not appear until after the basis of the permanent kidney is present (figs. 32, 33), and its relatively small size seems to indicate that it is never an important excretory organ in this animal. I have not observed the destiny of these excretory cells in *Fulgur*, but in *Crepidula*, where they are relatively larger, they are ultimately pinched off and set free with their load of nitrogenous waste. In *Fasciolaria*, as Glaser (1905) has shown, the external kidneys become colossal organs, which are filled with nitrogenous waste substance. Here the great size of these organs is probably associated with high metabolism of nitrogen, the result in this case of the cannibalism of the embryo. The small size of these organs in *Fulgur* probably indicates low nitrogenous metabolism in the embryo.

The definitive kidney has no structural connections with the larval ones. It appears as a single structure near the gastral end of the intestine (figs. 32 *et seq.*), and a duct develops from it which runs along the right side (left in ventral view) of the intestine (figs. 34, 35). As a result of the invagination which forms the mantle chamber, both the kidney and the intestine come to lie in the roof of that chamber, and since the kidney lies near the edge of the mantle it undergoes little movement during this invagination; the intestine, which lies farther from the mantle edge, moves under the kidney during this invagination, and thus the relative positions of these two organs are interchanged, the kidney coming to lie on the left side of the intestine (fig. 36).

10. GILL AND HEART.

At its earliest appearance the gill lies on the anterior side of the kidney and in close contact with it (figs. 33, 34). Before the formation of the mantle chamber it is merely an aggregation of cells and shows no characteristic structure. After the formation of the mantle chamber it lies on the left side of the kidney and its anterior border becomes crenated, each of these lobules giving rise later to a tentacle-like process.

The larval heart (figs. 35, 36, *L.H.*) appears as a blister under the blastoderm at the right of the larval kidney (fig. 34). The walls of this vesicle contain smooth muscle cells and are pulsatile. I have observed no definite vessels leading to or from this heart, and it probably serves merely to keep lymph moving through irregular channels. In later stages the larval heart is carried up on to the dorsal side of the embryo behind the head vesicle and velum (fig. 36); this movement is a part of the general twisting of all the organs, which were originally posterior to the foot.

I have not observed the manner of origin of the definitive heart and have not been able to recognize its anlage, unless it may be the dark body between the reference letters *M* and *Pa* in fig. 36.

IV. CONCLUSIONS.

The development of the principal organs and organ systems of *Fulgur* has now been described, with especial reference to their cell-lineage and topographical relations. It has not been possible to trace the entire cell-lineage of any organ, owing to the very large number of cells which are present before organs appear, but it is possible to determine the quartet and usually the individual cell of the quartet from which any organ arises. In a few cases (cerebral ganglion, velum, intestine) organs may be traced back to individual cells of a stage much later than the quartet formation, but in no case is it possible to observe every division of the cells which enter into the formation of an organ. This lack of a complete knowledge of the cell-lineage is not peculiar to *Fulgur*, but is general among forms in which the cell-lineage has been studied, and it is not a great hindrance to the study of the localizations of morphogenetic substances and processes of the egg. If groups of cells which give rise to certain organs can be traced back to certain quartet cells, this is usually sufficiently detailed information as to the cellular origin of an organ. In *Fulgur* it is unusually easy to trace this connection between blastomeres and organs, owing to the fact that the organs appear while the blastoderm is still a flat plate.

Next to the resemblances between *Fulgur* and *Crepidula* in the early cleavages, there is no similarity between these forms more striking than that which is found in the cellular origin of homologous organs. Although the organs of these two genera may differ widely in size and early position, there is not a single instance in which there is any good reason for supposing that these organs have arisen from unlike cleavage cells of the early stages. On the other hand, there is the best of evidence that homologous organs in *Crepidula* and *Fulgur* arise from corresponding cells of the different quartets, and even from corresponding cells of much later stages (60-cell stage), even though in still later stages these organs may occupy widely different regions of the embryo, as in the case of the cerebral ganglia.

With regard to the later cleavages, it is not only impossible to follow the lineage of individual cells until they give rise to organs, but it is certain that the cells of these two genera cannot be individually compared, since there are many more cells in *Fulgur* at a given stage of differentiation than in *Crepidula*. For example, there are about 250 cells in the embryo of *Crepidula* at the time of the first appearance of the shell gland, in *Fulgur* there are about 1,000 cells, and in any comparison of the cells at this stage it must be remembered that in general four cells of the latter are equal to one of the former. Not only does the number of cells differ in corresponding stages of differentiation in these two forms, but even in the same species there are variations and irregularities in the later cleavages which are not present in the earlier ones. When these irregularities of the later cleavages are compared with the invariable features of the early ones, such as the segregation of the ectoderm in three quartets of cells and the origin of the mesoderm of the trunk region from one cell of the fourth quartet, it is impossible to avoid the conclusion that the early cleavages are of greater morphogenetic value than the later ones. Furthermore, the conditions found in *Fulgur*, as compared with those in *Crepidula*, show that neither the presence of a large amount of yolk in the egg before cleavage, nor the modifications of the later stages, due to the presence of this yolk, affect the localization of the morphogenetic materials and processes in the earlier stages. The conclusion seems to be justified that the type of this localization is a more general and fundamental character than the form of gastrulation, or any other relatively late process in the ontogeny.

In *Fulgur* as in many other animals the egg shows polar differentiation while it is still in the ovary, and even before yolk formation begins. The yolk is laid down in all parts of the egg, and before maturation one pole is not noticeably richer in protoplasm than another. With the maturation of the egg the segregation of the protoplasm and yolk begins, most of the protoplasm passing to one pole and becoming the future ectoderm, while the yolk remains at the opposite pole and with a relatively small amount of protoplasm gives rise to the endoderm. In the first two cleavages this yolk is distributed equally to the four quadrants of the egg, and since the blastopore forms at the vegetal pole, the ectoderm must extend equally (but not synchronously) over the yolk in all directions. In the early stages of this overgrowth the anterior portion of the blastoderm extends more rapidly than the posterior portion, and by this means the cerebral and buccal ganglia are brought into close relation with the other organs present in the posterior margin of the blastoderm.

In many animals the yolk is not distributed equally to the four quadrants of the egg (*e.g.*, *Urosalpinx*, *Nassa*, *Dentalium*, *Chœtopterus*, etc.), and wherever this is the case a "yolk lobe" of considerable size is present. When one macromere is much larger than the others a large yolk lobe is attached to it; when the cleavage is approximately equal the yolk lobe, if present, is small. Crampton (1896) has shown that the removal of the yolk lobe causes the absence of mesoderm in *Illyonassa*; and in his beautiful "Studies on Germinal Localization" Wilson (1904) has found that when the polar lobe of *Dentalium* is removed the resulting larva lacks the post-trochal region and probably also the mesodermal bands. Both of these authors found that following the removal of the lobe the four quadrants of the egg were equal in size. It cannot be assumed that the size of the lobe is proportional to the size of the somatoblasts *2d* and *4d*, or to the size of the post-trochal region, or to the quantity of mesoderm which is formed later. In *Fulgur*, *Crepidula* and all other cases in which the quadrants are nearly equal in size, the yolk lobe is much smaller than the post-trochal region or the mesentomere *4d*, or even the mesodermal bands; while in *Illyonassa*, *Tritia*, and other forms in which one of the macromeres is much larger than the others, the yolk lobe is much larger than the cell *4d*. On the other hand the lobe is usually associated with unequal division of the yolk, and its size is proportional to that inequality. Therefore I believe that the real significance of the yolk lobe is to be found in its relation to equal and unequal cleavage.

Since the blastopore forms at the vegetal pole in all gasteropods, so far as known, unequal division of the yolk must lead to unequal growth on the part of the blastoderm; if the posterior macromere is large the overgrowth must be greater in a posterior direction than in any other; if the anterior macromere is larger than the others, as in certain opisthobranchs, the blastoderm must grow more anteriorly than posteriorly. When all the macromeres are equal the overgrowth must take place equally in all directions, though the case of *Fulgur* shows that it may be accelerated at one time in one portion of the blastoderm and at another time in another portion.

The most striking difference between *Fulgur* and other gasteropods is found in the manner of this overgrowth, and consequently in the manner in which the different organ bases are carried from their point of origin to their definitive positions in the embryo. In both the point of origin and the definitive position of the various organs there is fundamental agreement between *Fulgur* and *Crepidula*, but in the manner of passing from their point of origin to their final position there is marked dissimilarity. In *Crepidula* the region of greatest growth of the blastoderm lies on the dorsal side between the anlagen of the cerebral ganglia and the shell gland; in *Fulgur* it lies anterior to the cerebral anlagen. In *Crepidula*, as a result of this condition, the cerebral ganglia are carried forward around the anterior end of the embryo until they come to lie anterior to the mouth on the ventral side. In *Fulgur* the same ganglia are carried backward around the yolk until they finally reach the same position on the ventral side of the embryo.

It has been customary hitherto to regard such peculiarities of the embryo as primary, and the associated peculiarities of cleavage stages as secondary adaptations to these later appearing peculiarities; but this puts the cart before the horse. The adaptations of cleavage are adaptations to conditions pre-existing in the egg, and not adaptations to conditions which appear later in the embryo or adult. Given the enormous yolk of *Fulgur* or the small yolk of *Crepidula plana*, and the embryo must adapt itself to these conditions of the egg; or, in other words, the earlier conditions in ontogeny stand in the relation of cause to the later conditions, and not the reverse.

When I first observed in *Fulgur* the great modifications in the location of organ bases, which is unlike anything hitherto described, and found, for example, that the cerebral ganglia and velum were located in the posterior lip of the blastopore, I thought, for a time, that here was a new pattern of germinal localization, and that the generalization that homologous structures always come from homologous regions of the egg had broken down. Further study has shown that this is not the case, and that the great modifications in the location of embryonic organs in *Fulgur* are not primary but secondary, while the localization pattern in the early cleavages is the same as in other gasteropods.

Hence I regard the case of *Fulgur* as a triumph for the method and doctrine of cell-lineage. Those who see in this method only 'the counting of cells,' 'mitotic book-keeping,' 'the drudgery of dull minds,' have missed the whole point and significance of this method, which is not to name every cleavage cell, but to determine in what areas of the egg certain morphogenetic processes are located. To know that such processes may be localized in the egg is valuable information, even though the pattern of this localization should differ for every animal; but to have discovered that through all the multifarious modifications which are found in the embryos and adults of great animal classes, such as the gasteropods or annelids, this same pattern runs unchanged,—this is illuminating.

Summary.

1. The eggs of *Fulgur carica* are among the largest of gasteropod eggs, their relatively great size being due almost exclusively to the great quantity of yolk which they contain. These eggs are thirteen times the diameter and about 2,000 times the volume of those of *Crepidula plana*, with which particularly they are compared.

2. The cleavage of the egg of *Fulgur* is, cell for cell, like that of *Crepidula* up to the 56–60-cell stage, the only difference being in the

relative sizes of the macromeres in these two genera. Some of the individual features in which the cleavage of *Fulgur* resembles that of *Crepidula* are the following:

a. The direction, rhythm and quality of each cleavage is the same.

b. The first and second cleavages are equal; the first is transverse to the median plane of the embryo, the second coincides with that plane; a small yolk lobe is formed during each of these cleavages.

c. The ectoderm is segregated in three quartets of micromeres.

d. The first formed member of the fourth quartet, 4*d*, is the mesentomere; its anterior portion gives rise to the mesodermal bands and its posterior portion to the intestine.

e. The remaining members of the fourth quartet, viz., 4*A*, 4*B*, 4*C* are entomeres and give rise to all portions of the alimentary canal and its outgrowths, save the intestine and stomodæum.

f. The first quartet of ectomeres forms a "cross", with its centre at the apical pole and with anterior, posterior, right and left arms. Between the arms are the "turret" cells, and between these and the apical pole are the "rosette" cells; all of these cells are derived from the first quartet, except the "terminal" cells of the arms which are derived from the second quartet. The cross is formed of exactly the same cells in *Fulgur* as in *Crepidula,* but it is neither so distinct in form nor so persistent.

g. The second and third quartets surround the first and are composed of cells which divide in the same order and direction as in *Crepidula.*

3. In later cleavages many more ectoderm cells are formed in *Fulgur* than in *Crepidula* at corresponding stages of differentiation.

4. The overgrowth of the yolk is highly peculiar in *Fulgur.* By very great extension of the anterior half of the blastoderm, while the posterior half remains relatively fixed, all the organ bases are carried to the posterior margin of the blastoderm, where they form a kind of germ ring. Subsequently the posterior margin also moves over the yolk, so that the blastopore is finally formed at the vegetal pole.

5. Before the extension of the anterior portion of the blastoderm an apical invagination of ectoderm cells is formed in the region of the anterior arm of the cross. This invagination subsequently flattens out and completely disappears. Its function seems to be connected with the rapid extension of this part of the blastoderm.

6. The cerebral ganglia arise on each side of the apical invagination, probably from the anterior "rosette" cells. The velar cells arise around the outer margins of the ganglia, probably from the anterior

"turret" cells. By the rapid growth of the anterior portion of the blastoderm these organ bases are forced far apart and posteriorly until they come to lie in the posterior margin of the blastoderm, and by a continuation of this movement they are carried around to the ventral side of the embryo, where the two halves of these organs approach each other and finally unite in front of the mouth.

7. The paired buccal ganglia arise lateral to the cerebral and probably from derivatives of 2*a* and 2*c*; they extend over the yolk in the same manner as the cerebrals and finally concresce on the ventral side of the embryo posterior to the mouth.

8. All other organs (shell gland, pedal, pleural, parietal and abdominal ganglia, foot, intestine, kidney, gill, heart, etc.) arise from the median posterior portion of the blastoderm, and chiefly, if not entirely, from the two "somatoblasts," 2*d* and 4*d*. The bases of all of these organs, except the last three, appear when the blastoderm is still a small plate, and in the case of paired organs they are from the first connected across the median plane and do not, therefore, undergo concrescence. The origin of these post-oral organs from the two somatoblasts is strikingly like the condition found in annelids, and the elongation of the body of the gasteropod through the shell gland is similar to the elongation of the body of the annelid.

9. All homologous organs arise from corresponding cleavage cells in *Fulgur*, *Crepidula*, and probably all other gasteropods. Great increase in yolk does not modify the type of germinal localization, though it does profoundly modify gastrulation and later stages.

10. This indicates that germinal localization in the egg and in the cleavage stages is more fundamental and primitive than are the later processes of ontogeny.

CYTOLOGICAL.

11. During quartet formation the nuclei of the macromeres divide at relatively short intervals and they remain relatively small, the ratio of maximum nuclear diameter to cell diameter ("Kernplasma-relation" K/p) being 1 : 24. In later stages the resting period of the nucleus is very long and the ratio rises to 1 : 12. The size of the nucleus is therefore dependent not only upon the size of the cell, but also upon the length of the resting period. There is in this species no fixed ratio of nuclear size to cell size, and the cause of cell division cannot be found in the maintenance of a constant ratio.

12. After the formation of the fourth quartet the macromeres do not again divide, although the nuclei do. In the first and second cleavages the macromeres divide equally; this apparent anomaly is

probably due to the fact that cytoplasm is distributed through the yolk in the early stages and is lacking in the later ones. This suppressed cleavage of the yolk in the later stages is an approach to the condition found in meroblastic eggs.

References.

No attempt is made in this paper to review recent important contributions on cell-lineage, foremost among which must be mentioned the important memoirs of Robert (1903) on Trochus, Wierzejski (1905) on Physa, and Carazzi (1906) on Aplysia. The papers here listed are those mentioned in the body of this work.

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Reference Letters.

A, B, C, D, Macromeres.
1a, 1b, etc., First quartet cells.
2*a*, 2*b*, etc., Second quartet cells.
3*a*, 3*b*, etc., Third quartet cells.
4*d*, Mesentoblast.
4*A*, 4*B*, 4*C*, Secondary macromeres.
A.I., Apical invagination.
Bp., Blastopore.
Cb., Cerebral ganglion.
Bc., Buccal ganglion.
Pl., Pleural ganglion.
Pd., Pedal ganglion.
Pa., Parietal ganglion.
Ab., Abdominal ganglion.
E., Eye.
G., Gill.
H.V., Head vesicle.
I., Intestine.
K., Kidney.
Ex.K., Larval kidney.
L.H., Larval heart.
L.M., "Larval mesoderm."
M., Mantle.
Ms., Mesodermal bands.
O., Otocyst.
P.G., Pedal gland.
Sh., Shell gland.
T., Tentacle.
V., Velum.
Cm.M., Columellar muscle.
Oe., Œsophagus.

Description of Plates XXIII—XXVIII.

All the figures were drawn at the stage level with the aid of the camera lucida; plates XXIII, XXVII and XXVIII under Zeiss Apochromatic Obj. 16 mm., Oc. 4; plates XXIV, XXV and XXVI with Obj. 8 mm., Oc. 4. The figures of the former plates are therefore magnified 62 diameters; of the latter, 124 diameters. In the process of reproduction the figures were reduced a little more than half, so that as they appear on the plates they represent a magnification of about 30 diameters and 60 diameters respectively. All the drawings are of eggs or embryos of *Fulgur carica*, fixed, stained and mounted as described in the first section of this paper.

Plate XXIII.—Entire eggs; 1-cell to 24-cell stages.

Fig. 1.—Unsegmented egg, showing first cleavage spindle and surrounding cytoplasm.

Fig. 2.—Two-cell stage, showing læotropic turning of cytoplasm. nuclei and spheres preparatory to the second cleavage.

Fig. 3.—Four-cell stage; the positions of nuclei and spheres indicates that the transverse furrow is here the second one.

Fig. 4.—Eight-cell stage, showing first quartet of micromeres which have turned in a dexiotropic direction; in two of the macromeres are nuclear spindles for the formation of the second quartet, which arises in a læotropic direction.

Fig. 5.—Sixteen-cell stage. The first quartet has divided læotropically, giving rise to the "apical" cells centrally and the small "turret" cells peripherally. The second quartet cells are dividing in two quadrants, and the third quartet cells are arising dexiotropically from the micromeres.

Fig. 6.—Twenty-four-cell stage, resulting from the completion of all the divisions initiated in the preceding figure.

Plate XXIV.—Blastoderms during the earlier stages of cleavage.

Fig. 7.—Same stage as shown in fig. 6, but magnified twice as much; twenty micromeres and four macromeres; the separation of the ectoderm from the macromeres is now complete.

Fig. 8.—Forty-five-cell stage. Every cell of the preceding stage has divided, or is dividing, except the macromeres *A*, *B* and *C*. The "apical" cells have given rise to the "basals" of the arms of the cross; one second quartet cell in each quadrant has produced the "tip" cell (stippled) of each arm; the third quartet cells are dividing læotropically, and the macromere *D* has produced the mesentoblast cell, 4*d*.

Fig. 9.—Fifty-five-cell stage. All divisions initiated in the preceding figure are here completed, and in addition the basal cells of the transverse arms of the cross have divided. The mesentoblast (4*d*) has given rise to six cells (two large and four small ones), and the macromeres *A*, *B* and *C* have given off the other members of the fourth quartet, viz., 4*A*, 4*B* and 4*C*.

Fig. 10.—Fifty-six-cell stage. Similar to the preceding, but showing the basal cell of the anterior arm of the cross divided and spindles in some of the cells derived from the second quartet. The cross is shown in heavy outline; there are three cells in each of the arms except the posterior one. The centre of the cross is here, and elsewhere, marked by a cross line, the "tip" cells of the arms, by stipples.

Fig. 11.—Irregular 81-cell stage; derived from preceding stage by division of the basal cell of the posterior arm, of eight cells of the third quartet, of eight cells of the second quartet, and of eight turret cells.

Fig. 12.—Eighty-five-cell stage; derived from the preceding by the completion of all divisions there indicated and by the subdivision of the basal and middle cells in the transverse arms of the cross.

PLATE XXV.—Blastoderms, showing later stages of cleavage.

Fig. 13.—Another 85-cell stage, consisting of 72 ectomeres, 6 mesentomeres and 7 entomeres.

Fig. 14.—Stage with 111 cells; 98 ectomeres, 6 mesentomeres and 7 entomeres. This is a more advanced stage than the two following ones.

Fig. 15.—Stage with 89 cells; 76 ectomeres, 6 mesentomeres, 7 entomeres.

Fig. 16.—Stage with 104 cells; 91 ectomeres, 6 mesentomeres, and 7 entomeres.

Fig 17.—Stage with 121 cells; 104 ectomeres, 10(?) mesentomeres and 7 entomeres.

Fig 18.—Stage of about 320 cells. The ectomeres are very numerous and are somewhat depressed below the general level in the region anterior to the apical pole. The nuclei of the secondary macromeres, 4*A*, 4*B* and 4*C*, are dividing.

PLATE XXVI.—Blastoderms, from the time of formation of the apical invagination to the appearance of the primordia of definitive organs. All nuclei and cell boundaries, where shown, were drawn with the camera lucida, so that their number and location are fairly accurate.

Fig. 19.—Stage of approximately 512 cells. The apical invagination (*A.I.*) is a deep pit just anterior to the animal pole. The mesentoblast (4*d*) has given rise to the mesodermal bands (*Ms.*).

Fig. 20.—Stage slightly more advanced than the preceding, showing the apical invagination as a tubular ingrowth, with its opening near the anterior edge of the blastoderm; the nuclei of the secondary macromeres (4*A*, 4*B* and 4*C*) are dividing a second time.

Fig. 21.—Stage immediately after the flattening out of the apical invagination, only a trace of which is here shown. On each side of the latter is a group of cells, lying beneath the surface of the blastoderm, and represented with solid black nuclei, which are probably "larval mesoderm" cells (*L.M.*); the superficial group of cells in this region ultimately gives rise to the cerebral ganglion (*Cb.*), while the aggregation of cells lying between and in front of the mesodermal bands (*Ms.*) is the primordium of the shell gland. Two of the secondary macromeres (4*A* and 4*C*) have, exceptionally, divided.

Fig 22.—In this and the following figures all traces of the apical invagination have disappeared; in other respects this figure is similar to the preceding one.

Fig. 23.—Stage of about 1,000 cells, in which the shell gland is plainly visible as a disk of cells, slightly depressed in the middle, and in which the primordia of the cerebral (*Cb.*), buccal (*Bc.*), pleural (*Pl.*), and pedal (*Pd.*) ganglia and of the intestine (*In.*) are recognizable as groups of cells. The row of nuclei on the outer side of each cerebral ganglion probably represents the primordium of the velum, while the more deeply staining area connecting the two cerebral ganglia across the apical pole probably corresponds to the "cephalic neural plate" of annelids. The nuclei of the secondary macromere (4*B*) are dividing a third time, but the cell body remains undivided.

Fig. 24.—Stage in all regards similar to the preceding, except that the blastoderm has grown larger. The nuclei in the macromeres have here reached their maximum size (cf. the nuclei of the same cells in figs. 7 and 10).

PLATE XXVII.—Entire eggs, showing stages from the appearance of organ primordia to the completion of the overgrowth of the yolk.

Figs. 25 and 26.—Stages similar to the one shown in fig. 24. The primordia of different organs are indicated by the closely stippled areas, the stipples representing nuclei.

Fig. 27.—Stage in which the blastoderm has extended through the growth of its anterior portion, over about one-third of the yolk. The organ primordia are confined to a small area in the posterior margin of the blastoderm. The nuclei of the macromeres are dividing.

Fig. 28.—Stage in which the blastoderm has overgrown about one-half of the yolk. Primordia of the following organs are shown: Cerebral ganglia (*Cb.*), velum (*V.*), buccal ganglia (*Bc.*), pleural ganglia (*Pl.*), pedal ganglia (*Pd.*), otocysts (between pleural and pedal ganglia), intestine (*In.*), parietal ganglia (on each side of intestine), and shell gland.

Fig. 29.—Stage in which about two-thirds of the yolk has been overgrown. In addition to the organ primordia already named, the foot is also visible between the two pedal ganglia.

Fig. 30.—Final stages in the overgrowth of the yolk. The blastopore (*Bp.*) is a relatively small area of yolk at the vegetal pole, still uncovered by the blastoderm. The secondary macromeres (4*A*, 4*B* and 4*C*) lie in the lips of the blastopore, and while they contain many nuclei their cell bodies are still undivided. The velum has begun to extend forward around the anterior side of the blastopore, while the buccal ganglia (*Bc.*) lie in its lateral borders. All the organ primordia named above are plainly visible, and in addition the abdominal ganglion may be seen on the left of the intestine.

Plate XXVIII.—Entire embryos, from the closure of the blastopore to the formation of the larva.

Fig. 31.—Embryo showing the blastopore very small. The margin of the shell gland has extended widely, while the area within this margin is covered by the embryonic shell (*Sh.*).

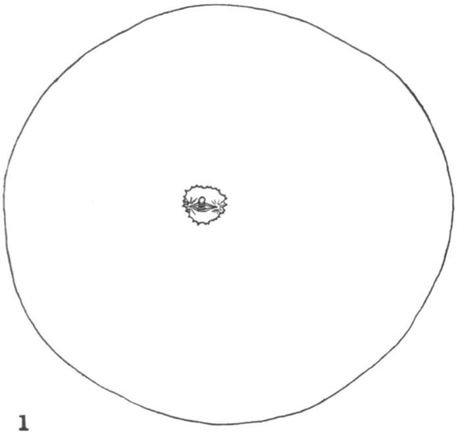
Fig. 32.—Stage showing the closure of the blastopore and the establishment of the pre-oral and post-oral velar bands The margin of the shell gland shows a deep notch posterior to the intestine, which grows more pronounced in later stages. The two cerebral ganglia are approaching each other in front of the blastopore. The primordium of the permanent kidney (*K.*) is recognizable.

Fig. 33.—Older stage, in which nearly one-half of the embryo is covered by the shell. The cerebral ganglia are united by a commissure in front of the mouth.

Fig. 34.—A stage in which about two-thirds of the embryo is covered by the shell.

Fig. 35.—Stage showing head vesicle (*H.V.*), larval heart (*L.H.*), mantle (*M.*), columellar muscle (*Cm.M.*), external kidneys (*Ex.K.*) and tentacles, (*T.*) in addition to the other organ primordia named in the description of figs. 28–30.

Fig. 36.—Later embryo, showing the formation of the mantle cavity, gill, velar lobes and the twisting of the nerve loop. The primordia of all important organs are present at this stage.



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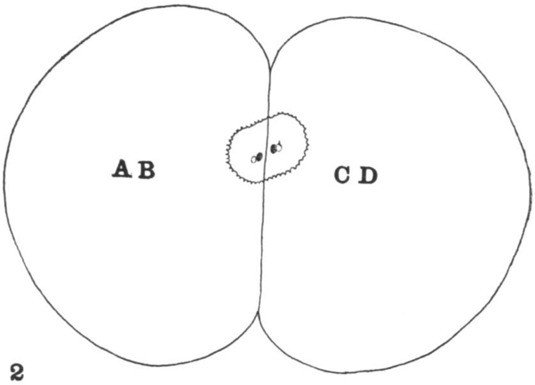

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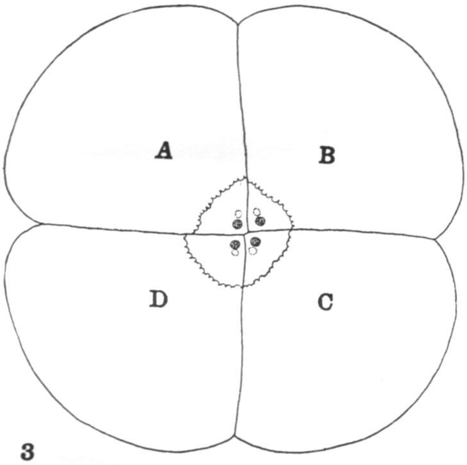

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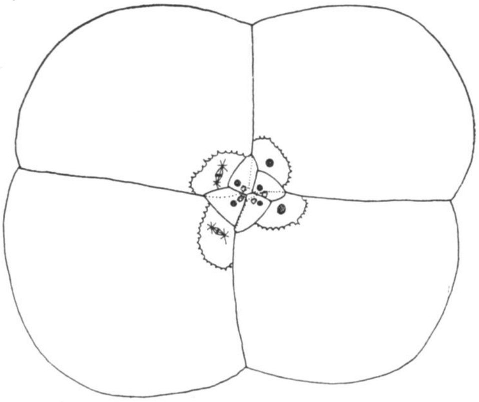

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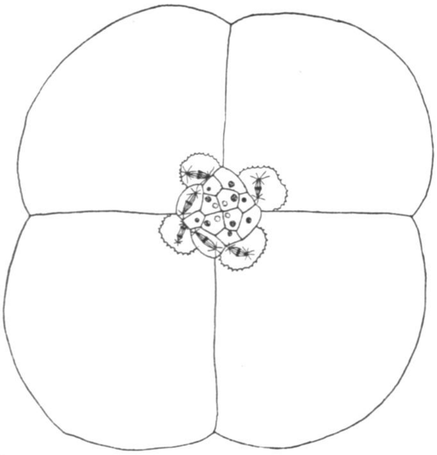

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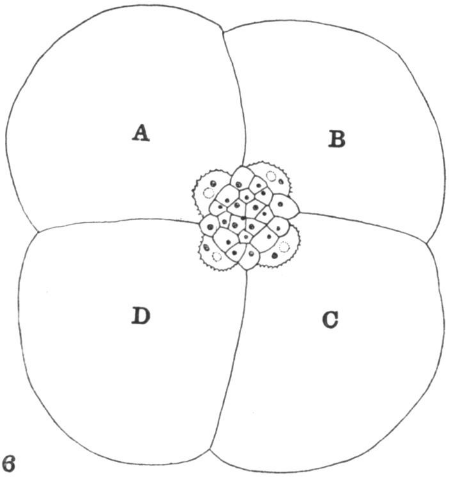

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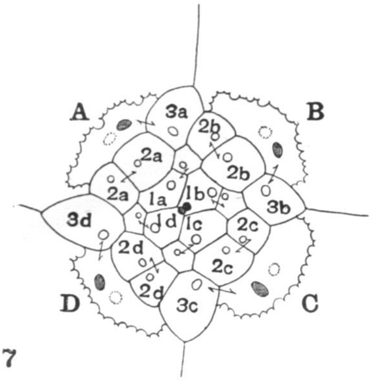

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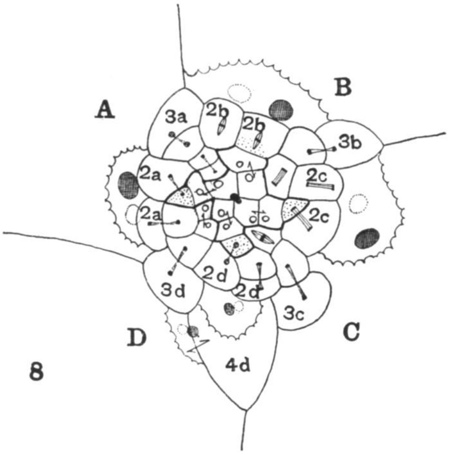

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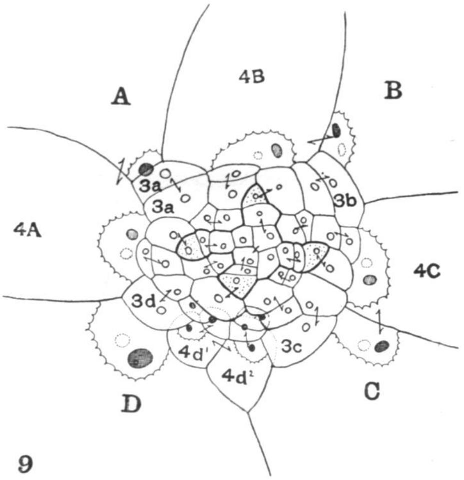

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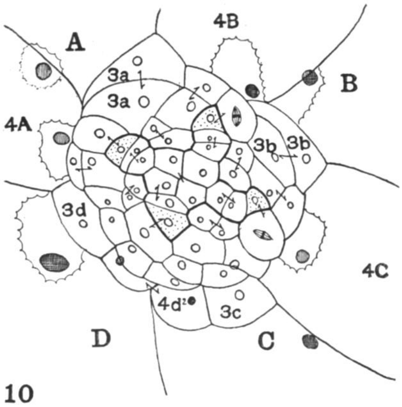

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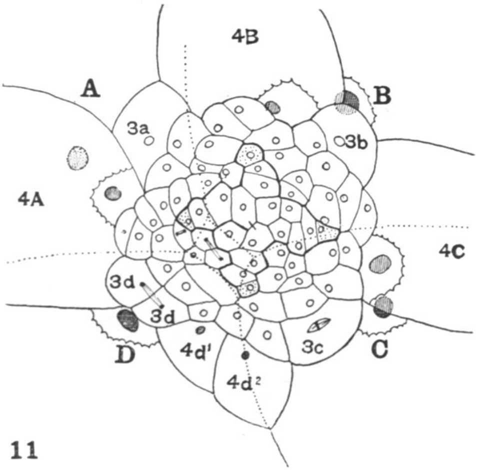

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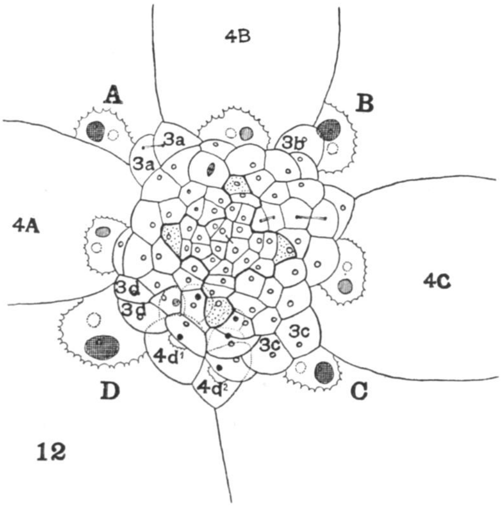

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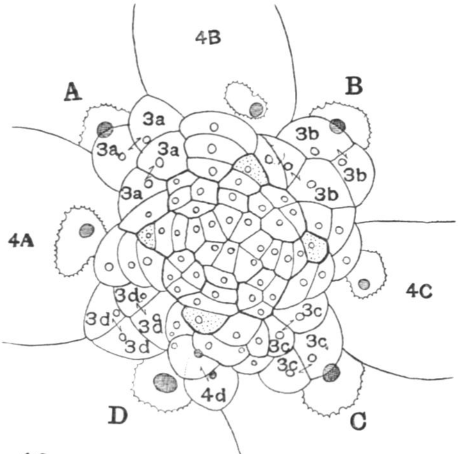

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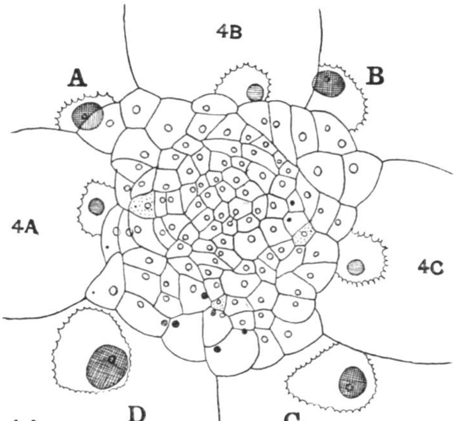

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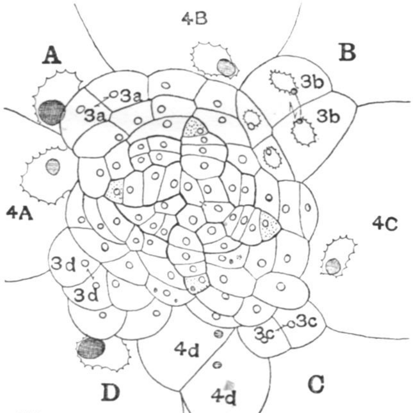

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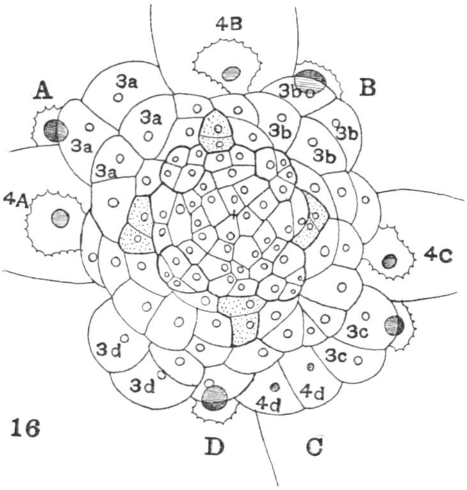

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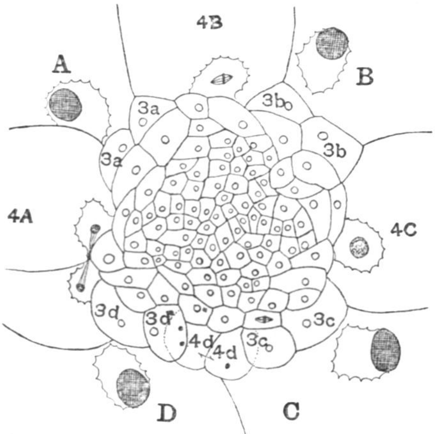

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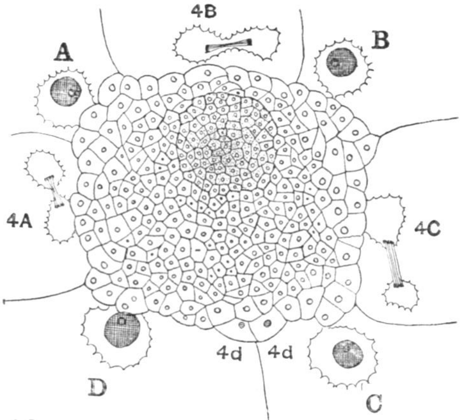

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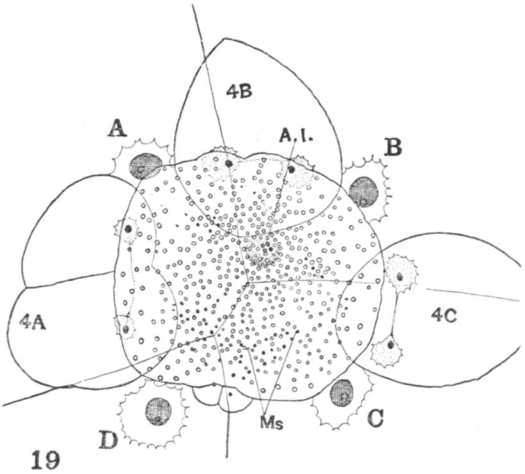

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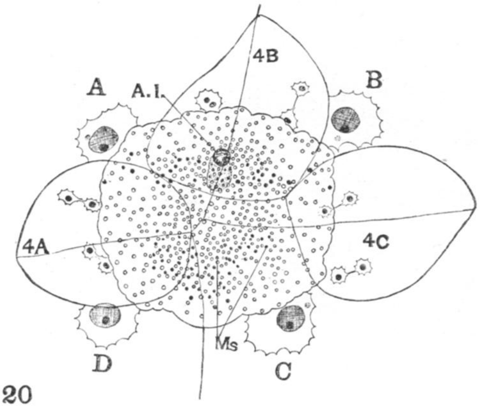

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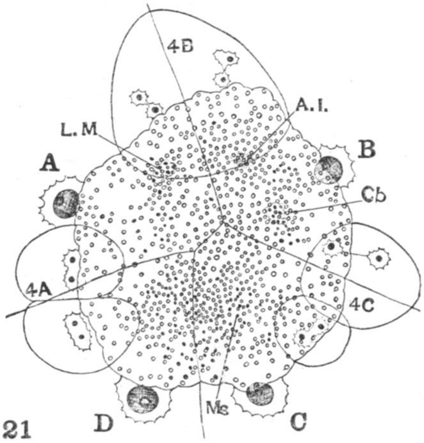

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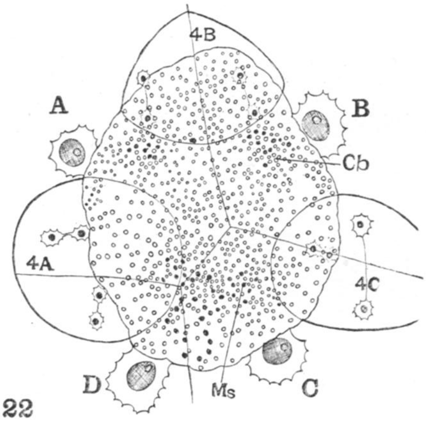

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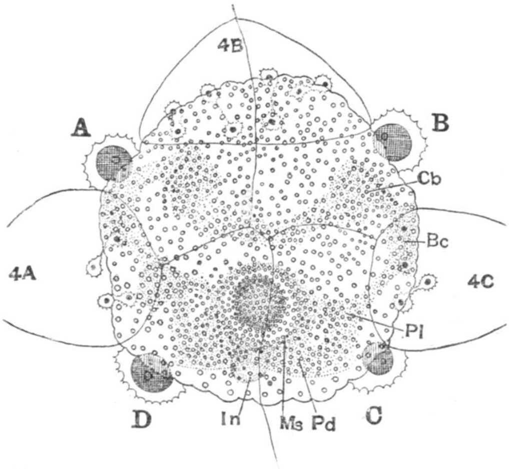

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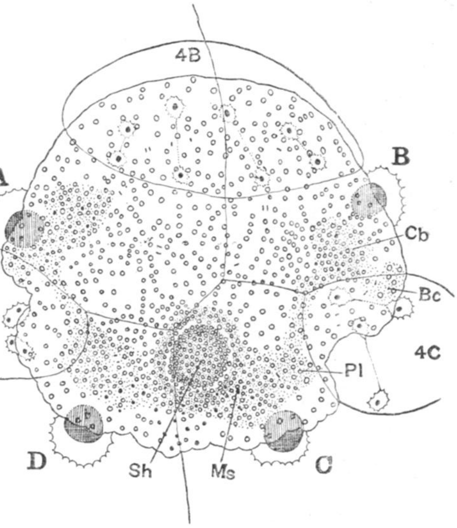

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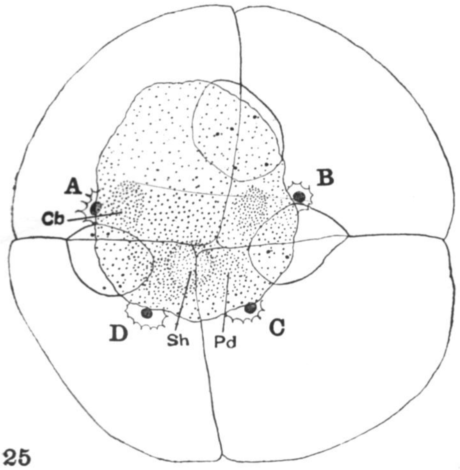

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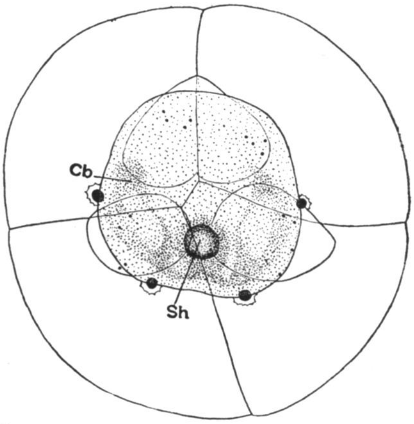

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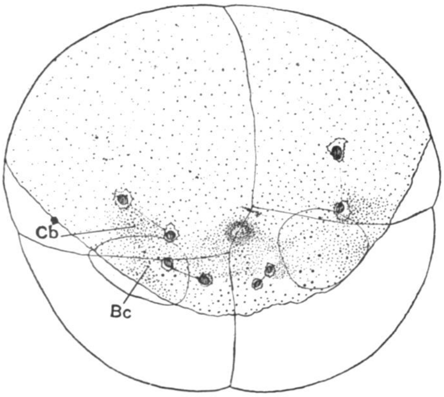

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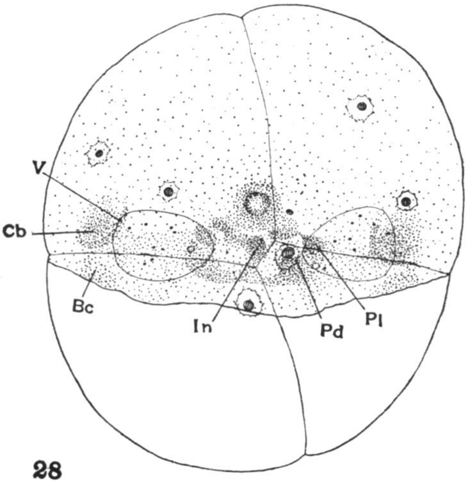

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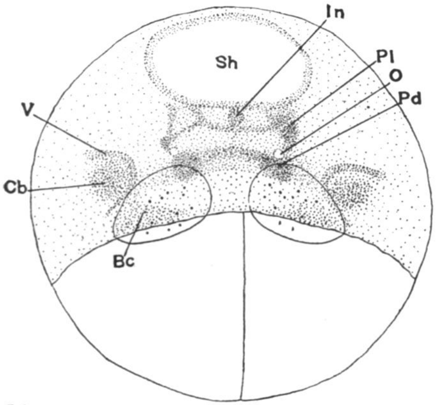

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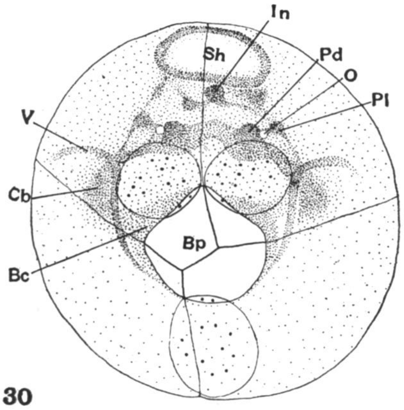

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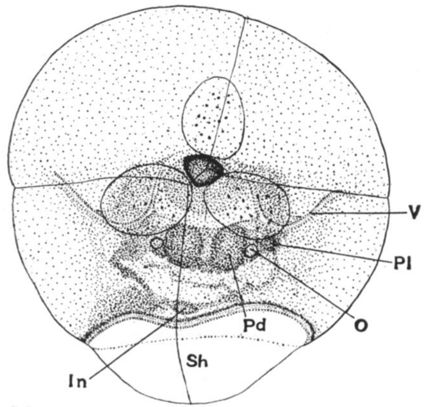

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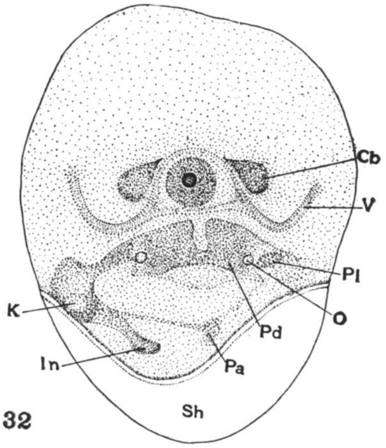

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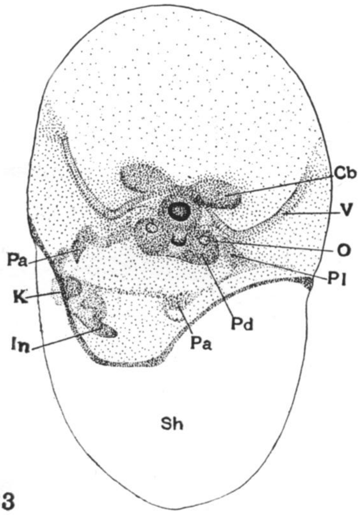

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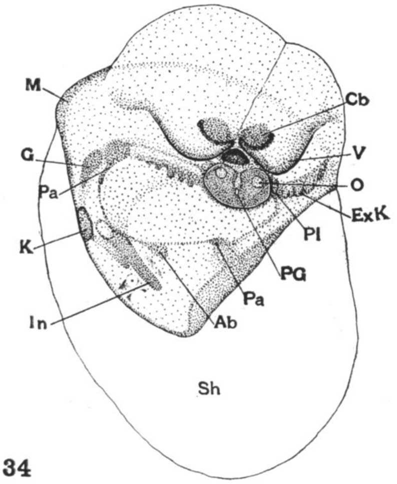

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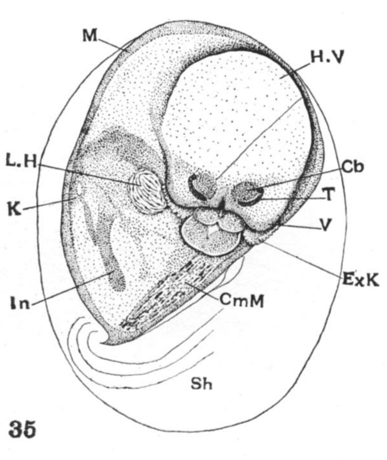

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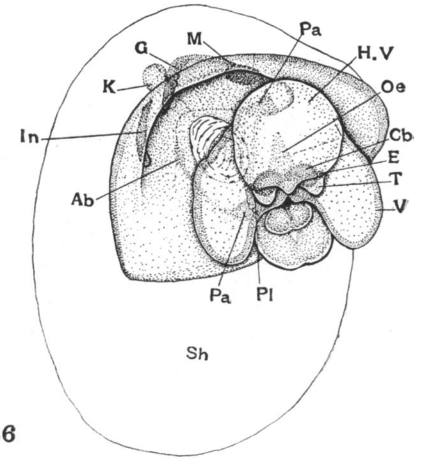

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